

# Morphometric and molecular characterization of populations of *Pratylenchus kumamotoensis* and *P. pseudocoffeae* (Nematoda, Pratylenchidae) newly recorded in Korea

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## Abstract

At least 70 species of root-lesion nematodes, *Pratylenchus* spp., have been identified worldwide, many of which are serious pests of various agricultural crop plants. In Korea, only 14 species have been recorded in vegetable and fruit fields. Here, we report two new records of root-lesion nematode species in Korea based on morphometric and molecular methods. Soil samples were collected from chrysanthemum cultivars in various regions. Morphometric diagnosis showed that two new records for Korea: *Pratylenchus kumamotoensis* in Chilgok County and *P. pseudocoffeae* in Geumsan County. In addition, molecular diagnosis using the two sequences of the internal transcribed spacer (ITS) and the D2–D3 region of ribosomal DNA showed that these two species were most similar with those from Japan, Costa Rica and USA. The similarities of the ITS and D2–D3 sequences were 99.85 and 99.74%, respectively, for *P. kumamotoensis* and 99.99 with Costa Rica populations and 99.86% with USA populations, respectively, for *P. pseudocoffeae*. To our knowledge, this is the first report of two species in Korea.

## Keywords

Chrysanthemum cultivars, ITS, Root-lesion nematodes

## Introduction

In 2012–2014, amphimictic root-lesion nematode populations were detected in soil and root samples from chrysanthemum (*Chrysanthemum* sp.) fields located in Chilgok and Geumsan Counties in Korea. The population from Chilgok Co. was identified as *Pratylenchus kumamotoensis* and the other from Geumsan Co. as *P. pseudocoffeae* using morphological and molecular analyses. The phylogenetic relationship of these Korean root-lesion nematodes with other populations reported on chrysanthemum in Japan and distant geographical areas was also analyzed.

Nematodes were extracted from the soil samples using Cobb's sieving and gravity method and the modified Baermann funnel method. Extracted nematodes in water suspension were killed by gentle heating, dehydrated by using the rapid lactophenol method and transferred in glycerin in permanent slides (Southey 1986). Nematode specimens were measured with the aid of Zeiss M1 light microscope. DNA from specimens of the two populations was extracted using the proteinase K method. Extracted DNA from a single nematode was directly used for PCR analysis.

The sequence of the ITS region, including ITS1, 5.8S, and ITS2, was amplified using the forward TW81 primer (5'-GTT TCC GTA GGT GAA CCT GC-3') and reverse AB28 primer (5'-ATA TGC TTA AGT TCA GCG GGT-3') (Tanha Maafi et al. 2003). The sequence of the D2–D3 expansion segment of 28S rDNA was amplified using the forward D2A primer (5'-ACA AGT ACC GTG AGG GAA AGT TG-3') and reverse D3B primer (5'-TCG GAA GGA ACC AGC TAC TA-3') (Subbotin et al. 2006). The PCR products were purified using Wizard SV Gel and PCR Clean-Up System (Promega, Madison, WI, USA) and cloned using T-blunt vectors and DH5 $\alpha$  competent cells, as described in the manufacturer's procedure (Solgent, Daejeon, Korea). The sequences were determined using the vector internal primer sets (M13F/M13R) at the Solgent Sequencing Facility (Daejeon, Korea) with a BigDye<sup>®</sup> Terminator Cycle Sequencing Kit and ABI 3730XL DNA analyzer (Applied Biosystems, Foster City, USA). The newly obtained sequences were assembled and edited by the alignment software Clustal W version 2.0 (Larkin et al. 2007; Goujon et al. 2010; McWilliam et al. 2013) and submitted to a search for similarity in GenBank using the Blast program (Altschul et al. 1990; Morgulis et al. 2008). Multiple sequence alignment was performed using Bioedit version 7.2.5 (Hall 1999), and a phylogenetic analysis was conducted using the MEGA 6 program (Tamura et al. 2013) with the neighbor-joining method (Saitou and Nei 1987). The GenBank data of related species were also included in the phylogenetic analysis.

The morphometric characters of the Korea population of *P. kumamotoensis* females that were measured included (n = 29) : body length =  $511.3 \pm 30.87$  (451.9 – 564.1  $\mu$ m), a =  $26.7 \pm 2.10$  (23.2 – 31.9  $\mu$ m), b =  $6.9 \pm 0.55$  (6.0 – 7.9  $\mu$ m), b' =  $5.0 \pm 0.40$  (4.3 – 5.8  $\mu$ m), c =  $20.3 \pm 1.87$  (17.2 – 23.9  $\mu$ m), c' =  $2.4 \pm 0.19$  (2.0 – 2.6  $\mu$ m), V(%) =  $75.7 \pm 1.85$  (71.4 – 81.8), stylet =  $14.8 \pm 0.55$  (13.4 – 15.7  $\mu$ m).



The morphometric characters obtained for the Korean *P. pseudocoffeae* females included ( $n = 36$ ): body length =  $565.9 \pm 51.99$  ( $475.3 - 660.4 \mu\text{m}$ ),  $a = 23.1 \pm 1.67$  ( $20.7 - 29.2 \mu\text{m}$ ),  $b = 6.8 \pm 0.46$  ( $6.1 - 7.7 \mu\text{m}$ ),  $b' = 3.7 \pm 0.24$  ( $3.2 - 4.1 \mu\text{m}$ ),  $c = 19.4 \pm 1.40$  ( $17.3 - 21.8 \mu\text{m}$ ),  $c' = 2.0 \pm 0.23$  ( $1.6 - 2.4 \mu\text{m}$ ),  $V(\%) = 79.6 \pm 0.80$  ( $78.2 - 81.2$ ), stylet =  $17.2 \pm 0.97$  ( $15.9 - 19.3 \mu\text{m}$ ).

These morphometrics matched those reported by Mizukubo et al. (2007) for *P. kumamotoensis* and Mizukubo (1992) for *P. pseudocoffeae* in Japan. Korean specimens of *P. kumamotoensis* showed the esophageal glandular lobe aberrantly overlapping dorsally the intestine like the paratypes described in Japan. Body and stylet length of Korean *P. kumamotoensis* were  $50 \mu\text{m}$  shorter and  $1 \mu\text{m}$  longer, respectively, than those of the Kanoya paratype of this species. Females of Korean *P. pseudocoffeae* had sub-hemispherical tail with smooth terminus like the paratypes from Japan. Stylet length and distance between the base of esophageal gland lobe to the anterior body end of Korean *P. pseudocoffeae* were  $1 \mu\text{m}$  longer and  $6 \mu\text{m}$  shorter, respectively, than those of the Miyazaki paratype and the populations from aster reported in Florida (USA).

The ITS regions, including ITS1, 5.8S, and ITS2 of the Korean *P. kumamotoensis* and *P. pseudocoffeae* were 664 and 849 bp, respectively (Suppl. material 1: Fig. S1, 2). In addition, the amplified D2–D3 regions of the two species were 762 and 737 bp, respectively (Suppl. material 1: Fig. S3, 4). The comparison of nucleotide sequences of PCR products with those of the GenBank database showed that both the ITS and D2–D3 sequences of the Korean *P. kumamotoensis* were most similar to those of *P. kumamotoensis* of Japan with 99.85 and 99.74% similarity, respectively. The ITS sequences of the Korean *P. pseudocoffeae* population were most similar to those of *P. pseudocoffeae* populations from Costa Rica, with 99.99% similarity (Araya et al. 2016), whereas the D2–D3 sequences were most similar to those of the population from USA, with 99.86% similarity (Duncan et al. 1999).

The results of the phylogenetic analysis using ITS sequences of our sample (Chilgok population) and eight populations of *P. kumamotoensis* from Kagoshima, Kumamoto, and Oita Prefectures of Japan showed that they clustered in two clades, which were not geographically separated (Suppl. material 1: Fig. S5). Namely, one clade included Kagoshima, Kumamoto, and Oita samples, but the other included Kumamoto and Oita samples. Our Chilgok sample (KT175515) was least different from the Oita sample (LC030318), with a 0.15% difference, but highly different from the Kagoshima sample (LC030312) with a 3.04% difference. In addition, the D2–D3 sequence of our Chilgok sample (KT175528) had a 0.26% difference with one listed sequence (JX144360) from Kumamoto (Suppl. material 1: Fig. S6). The results of the phylogenetic analysis using ITS sequences of our Geumsan sample (KT175523) and six populations of *P. pseudocoffeae* from Costa Rica, Iran and Japan (Araya et al. 2016; De Luca et al. 2011) showed that they differed by no more than 0.35%. Our Geumsan sample was 0.12% different from the Japan sample (LC030337) and 0.35% different from the Iran sample (FR692276) (Suppl. material 1: Fig. S5). Further, it was only 1 bp different from Costa Rica sample (KT971367). In addition, the D2–D3 sequence

of our Geumsan sample (KT175531) differed by 0.14% from the USA sequence (AF170444) and 0.27% from the Costa Rica sequence (KT971360) (Suppl. material 1: Fig. S6). All of the new sequences for the Korean populations of *P. kumamotoensis* and *P. pseudocoffeae* were deposited in GenBank with the accession numbers listed in parenthesis in the text.

To our knowledge, this is the first record of occurrence of *P. kumamotoensis* and *P. pseudocoffeae* in chrysanthemum fields in Korea.

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## Supplementary material I

### Analysis of ITS2 and D2-d3 segment sequences of *Pratylenchus kumamotoensis* and *P. pseudocoffeae*.

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Data type: Nucleotide sequences and cladograms

Explanation note:

Figure S1. Alignment of the ITS region of *Pratylenchus kumamotoensis*.

Figure S2. Alignment of the ITS region of *Pratylenchus pseudocoffeae*.

Figure S3. Alignment of the D2-D3 expansion segment of *Pratylenchus kumamotoensis*.

Figure S4. Alignment of the D2-D3 expansion segment of *Pratylenchus pseudocoffeae*.

Figure S5. Neighbor-joining tree for *Pratylenchus* species based on ITS sequences.

Numbers adjacent to branches denote the bootstrap values (>50%) of 1000 replicates. Sequences identified in this study were indicated in bold.

Figure S6. Neighbor-joining tree for *Pratylenchus* species based on D2-D3 sequences. Number adjacent to branches denote the bootstrap values (>50%) of 1000 replicates. Sequences identified in this study are indicated in bold.

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# Review of the western African millipede genus *Diaphorodesmus* Silvestri, 1896 (Diplopoda, Polydesmida, Chelodesmidae), with the description of a similar, but new monotypic genus from Cameroon

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## Abstract

The genus *Diaphorodesmus* is revised and shown to comprise only a single species, *D. dorsicornis* (Porat, 1894) by priority, with the only other formal congener, *D. attemsii* Verhoeff, 1938, considered as its junior subjective synonym, **syn. n.** A new monotypic genus, *Diaphorodesmoides* **gen. n.**, is created to include *D. lamottei* **sp. n.**, from southwestern Cameroon. Both these genera seem to be especially similar in sharing remarkable dorsal horns on metaterga 2–4, a unique synapomorphy in the basically Afrotropical subfamily Prepodesminae, family Chelodesmidae, to which they belong. In contrast to *Diaphorodesmus* which shows two, increasingly short, paramedian horns on each of metaterga 2–4, the ozopores borne on distinct porostoles, and the gonopod prefemoral process and solenophore less strongly elaborate, *Diaphorodesmoides* **gen. n.** has a single, increasingly large, central horn on each of metaterga 2–4, the ozopores opening flush dorsolaterally on the surface of poriferous paraterga, and both the gonopod prefemoral process and solenophore especially complex. The genus *Campodesmoides* VandenSpiegel, Golovatch & Nzoko Fiemapong, 2015, and its sole, and type, species *C. corniger* VandenSpiegel, Golovatch & Nzoko Fiemapong, 2015, are transferred from Campodesmidae to Chelodesmidae and formally synonymized with *Diaphorodesmus* and *D. dorsicornis*, both **syn. n.**

Keywords

Taxonomy, synonymy, new species, Cameroon, Nigeria, Equatorial Guinea

Introduction

The western African genus *Diaphorodesmus* Silvestri, 1896, was erected by Silvestri (1896) to encompass a single species that Porat (1894) had described as *Paradesmus dorsicornis* Porat, 1894, from Cameroon. The original description and most of the illustrations as presented by Porat (1894) were quite adequate for that time, showing almost all necessary details of body structure, including the remarkably strong, suberect, paramedian horns gradually decreasing in size on metaterga 2–4. The syntypes were said to be abundant, mostly taken at N’dian and Kitta. Only the gonopod was depicted too small and schematically, apparently this being one of the reasons for subsequent confusion.

Carl (1905), based on material from Cabo San Juan, then Spanish Guinea, now Equatorial Guinea, and, later, Attems (1931, 1938), based on rich samples coming from Mukonje Farm, Bibundi and Victoria, Cameroon, provided detailed descriptions and very clear illustrations of what they identified as *D. dorsicornis*.

Verhoeff (1938), having studied some more material of *Diaphorodesmus* from Cameroon, yet with neither the number of specimens nor any precise locality indicated, came to the conclusion that what Attems (1931) had taken for *D. dorsicornis* was actually a different species he named *D. attemsii* Verhoeff, 1938. In addition, he illustrated the gonopod of what he believed to be *D. dorsicornis* and, in a tabular form, also listed the main differences in body structure between the two species, as follows (translated from German).

<i>D. dorsicornis</i> Porat, Verh. The three pairs of dorsal processes on diplosomites 2–4 are similarly well-developed; that of the 4 <sup>th</sup> not displaced from the posterior edge. 4 <sup>th</sup> metatergite with 6 acute anterior tubercles, the two paramedian the largest.	<i>D. attemsii</i> Verh. Of the three rows of dorsal processes those on the 4 <sup>th</sup> segment are not only smaller than the others, but also completely removed from the posterior edge. 4 <sup>th</sup> metatergite with 4 projections, all about the same size.
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Besides this, Verhoeff (1938) created the subfamily Odontokrepinae (recte: Odontokrepidinae) Verhoeff, 1938, to harbour only two genera: *Diaphorodesmus* and *Odontokrepis* Attems, 1898. The latter genus was said to be distinguished from the former by the presence of tergal horns on segments 2–4, and of porosteles. Attems (1940) regarded *Odontokrepis* a dubious genus with three species from Cameroon, whereas Hoffman (1980) treated it as a junior synonym of *Anisodesmus* Cook, 1895, with three species from Liberia (!), and the subfamily Odontokrepidinae as a junior synonym of Prepodesminae Cook, 1896.

Hoffman evidently believed that Verhoeff (1938) had erred as well in regarding his sample as representing a true *D. dorsicornis*. He drew the gonopod of a syntype of *D. dorsicornis*, still kept in the Porat collection at the Naturhistoriska Riksmuseet in

Stockholm (NHRS), Sweden (Fig. 3A, B), and the gonopod of a ♂ from Victoria, Cameroon (housed in the Naturhistorisches Museum Wien (NHMW), Vienna, Austria) which Attems (1931, 1938) had identified as *D. dorsicornis* and which Verhoeff (1938) had assigned to *D. attemsii* (Fig. 3C, D). Hoffman also abundantly illustrated (Fig. 2) a ♂ from Port Harcourt, Rivers State, Nigeria (likely still housed in the Virginia Museum of Natural History where Hoffman worked), and assigned it to the species that Verhoeff (1938) had considered as a true *D. dorsicornis*. Although Verhoeff's (1938) sample from an unknown place in Cameroon was different from the ♂ from Port Harcourt, Hoffman provisionally referred both to a new species. As a result, Hoffman (1980), in the only published account of *Diaphorodesmus*, said that the genus contained three species from Cameroon.

The present paper has largely been prompted by the recent description of *Campodesmoides* VandenSpiegel, Golovatch & Nzoko Fiemapong, 2015, a monobasic genus that only encompasses the type-species, *C. corniger* VandenSpiegel, Golovatch & Nzoko Fiemapong, 2015, from Cameroon (VandenSpiegel et al. 2015). That genus was erroneously assigned to the endemic western African family Campodesmidae, but in fact both the genus and species are junior synonyms of *Diaphorodesmus* and *D. dorsicornis*, respectively, in the basically Afrotropical subfamily Prepodesminae Cook, 1896, family Chelodesmidae Cook, 1895.

To correct the mistake, we have been able to retrieve the unpublished relevant archives of the late R.L. Hoffman, housed in the Virginia Museum of Natural History, Martinsville, Virginia, U.S.A. In addition, we have gathered all relevant information concerning the type series of *D. attemsii*, kept at the NHMW. This, plus several, largely unpublished samples received for study from the collections of the Muséum national d'Histoire naturelle (MNHN), Paris, France, the Natural History Museum of Denmark (ZMUC), Copenhagen, Denmark, and the Bayerische Zoologische Staatssammlung (ZSM), Munich, Germany, has allowed us not only to finally clarify the tangled history of studies on *Diaphorodesmus*, but also to add a new genus and species described below.

## Material and methods

The material treated here derives from the collections of the Musée Royal de l'Afrique Centrale (MRAC), Tervuren, Belgium, the MNHN, the ZMUC, and the ZSM. The samples are stored in 70% ethanol. Specimens for scanning electron microscopy (SEM) were air-dried, mounted on aluminium stubs, coated with gold and studied using a JEOL JSM-6480LV scanning electron microscope. Photographs were taken with a Leica DFC 500 digital camera mounted on a Leica MZ16A stereo microscope. Images were processed with Leica Application Suite software.

In the species catalogue section, D stands for a description or descriptive notes (sometimes also including a key, discussion, new status, synonymy or combination), and R for new or old records.



## Results

**Class Diplopoda Blainville-Gervais, 1844**

**Order Polydesmida Leach, 1814**

**Family Chelodesmidae Cook, 1895**

**Genus *Diaphorodesmus* Silvestri, 1896**

*Diaphorodesmus* Silvestri, 1896: 197.

*Diaphorodesmus* – Cook 1896: 16; Attems 1899: 311; 1931: 91; 1938: 409; Carl 1905: 271; Verhoeff 1938: 166; Hoffman 1980: 155.

*Campodesmoides* VandenSpiegel, Golovatch & Nzoko Fiemapong, 2015, **syn. n.**

**Type species.** *Campodesmoides corniger* VandenSpiegel, Golovatch & Nzoko Fiemapong, 2015, by original designation.

**Type species.** *Paradesmus dorsicornis* Porat, 1894, by original designation.

**Diagnosis.** A genus of Prepodesminae, Chelodesmidae that is distinguished by the presence of conspicuous paramedian, increasingly short, dorsal, horns on metaterga 2–4, coupled with the normal pore formula: 5, 7, 9, 10, 12, 13, 15–19, the ozopores being borne on conspicuous porosteles; the spiracles are small and inconspicuous; and the gonopod telopodites suberect, *in situ* directed forward, held parallel to each other, not crossing mesally; prefemoral (= densely setose) part erect, taking up about 2/3 of total gonotelopodite length, without a femorite part, but with a prominent dorsal process (**pfp**), set off from acropodite by a distinct cingulum; acropodite clearly twisted, divided parabasally into one smaller dorsobasal lobule (**lo**) and two large lamellar lobes, the ventral lobe forming a solenophore (**sph**) to support a dorsal solenomere lobe (**slo**) with only an indistinct, small solenomere proper on top.

***Diaphorodesmus dorsicornis* (Porat, 1894)**

Figs 1–7, 12

*Paradesmus dorsicornis* Porat, 1894: 33, figs 3–3c (D).

*Diaphorodesmus dorsicornis* – Silvestri 1896: 197 (D) (erection and typification of *Diaphorodesmus*); Cook 1896: 16 (D); Attems 1899: 312, plate 7, fig. 167 (D) (reiterated original description and a reproduced original figure); 1931: 100, figs 147–151 (D, R); 1938: 409, figs 451–452 (D, R); Carl 1905: 271, plate 6, fig. 1–1a (D, R).

*Diaphorodesmus attemsii* Verhoeff, 1938: 167, figs 1–3 (D), **syn. n.**

*Diaphorodesmus attemsii* – Attems 1940: 560 (D, R).

*Campodesmoides corniger* VandenSpiegel, Golovatch & Nzoko Fiemapong, 2015: 2, figs 1–3 (D), **syn. n.**

**Material examined.** Apart from the type series of *Campodesmoides corniger*, deposited at MRAC (VandenSpiegel et al. 2015), the following unpublished samples are available.

1 ♂ (MNHN JB254), Cameroon, Kumba, 25.XI.1975, leg. M. Lamotte (*D. dorsicornis*, det. J.-P. Mauriès); 5 ♂, 2 ♀ (ZMUC), eastern Nigeria, Osomba 56 miles from Calabar, 17.VI.1965; 1 ♀ (ZMUC), eastern Nigeria, 1963, all leg. V. Schiøtz (*D. attemsii*, all det. H. Enghoff).

**Revised published material.** 1 ♂, 2 juveniles (fragments of caudal body part only) (ZSM Reg. No. A 20052425 + slide A 20035316), “Kamerun”, without further information (*D. dorsicornis*, det. K.W. Verhoeff).

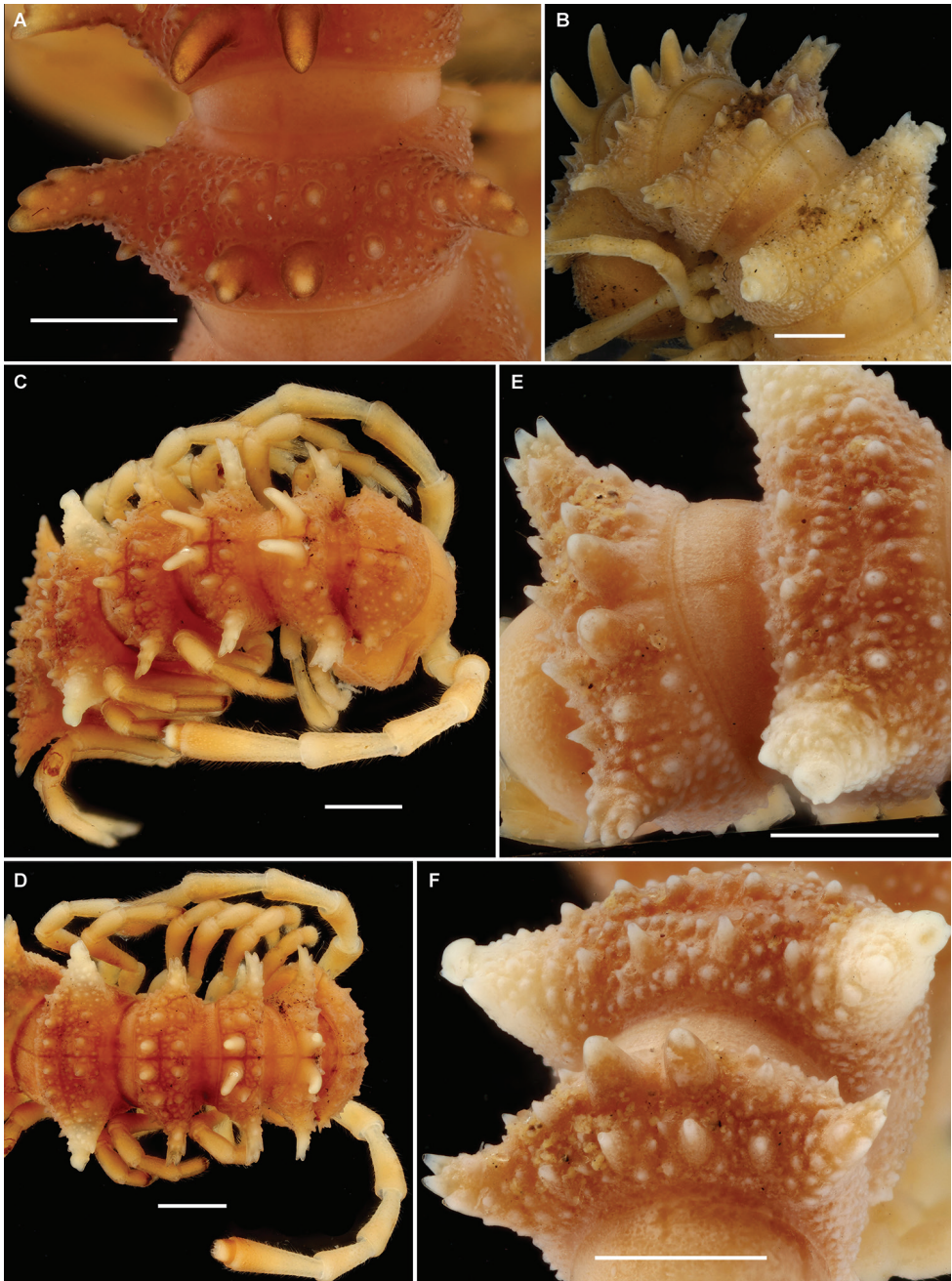
**Remarks.** This species enjoys several descriptions, the latest of which (VandenSpiegel et al. 2015) is particularly complete and detailed. We only add here more pictures and drawings (Figs 1–7) to show evident variations in some somatic and gonopodal characters that bridge *D. dorsicornis* and *D. attemsii* and justify their synonymization.

Considering the measured material published elsewhere (Porat 1894; Attems 1938; VandenSpiegel et al. 2015) and here, body size variations are quite considerable both between individuals and, to a lesser degree, sexes: length 26–35 mm (♂, ♀), width of midbody pro- and metazonae 2.1–3.5 and 3.0–4.9 mm (♂) or 2.5–3.6 and 3.6–5.0 mm (♀), respectively. General coloration varies from yellow through grey-brown to blackish (Porat 1894; Carl 1905; Attems 1931, 1938; VandenSpiegel et al. 2015).

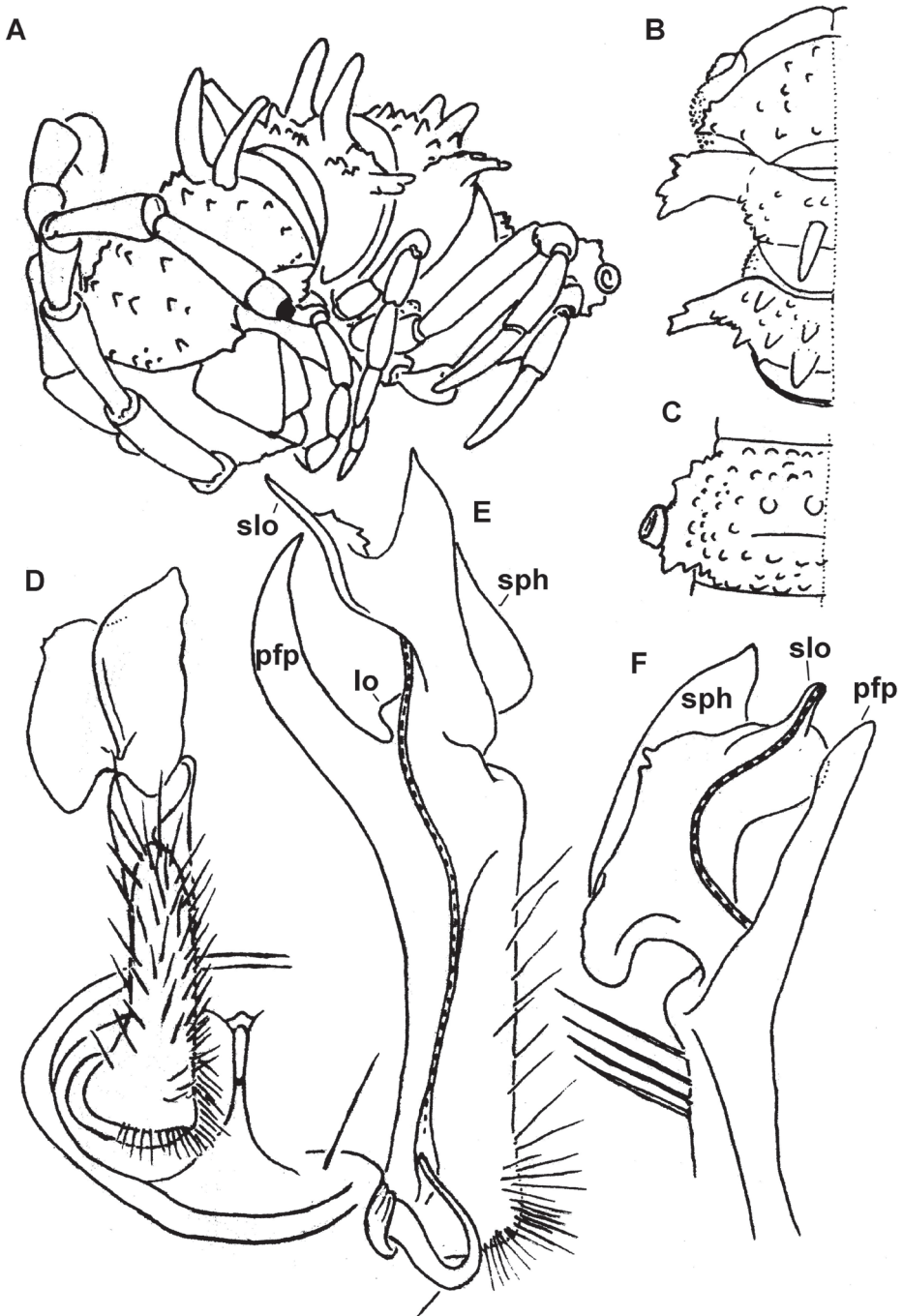
As regards the somatic characters mentioned by Verhoeff (1938) and quoted above that distinguish *D. attemsii* from *D. dorsicornis*, they are actually mistaken or reflecting individual variations. Thus, the dorsal horns on metaterga 4 are typically somewhat shorter in the ♀ compared to the ♂, and they tend to be more or less gradually and increasingly reduced from metatergum 2 to 4 in both sexes. The higher the horns on metatergum 4, the less strong their shift forward off the caudal margin. This shift is usually particularly apparent in the ♀.

The more or less evident cones in front of these horns are usually subequal in shape and size, 2+2, arranged in a transverse row (Fig. 1A, C, D). However, occasionally there are variations observed in shape and size of those cones as well. The pertinent material of Verhoeff (1938), at least the single adult ♂ at his disposal which is currently kept at the ZSM, shows the typical 2+2 (not 3+3!) cones, albeit the central pair is indeed a little larger than the lateral one, while the dorsal horns are relatively short, tuberculiform, clearly set off from the caudal margin of the metatergum (Fig. 1E, F). The gonopod structure of the ZSM ♂ is likewise closer to the one as depicted by Attems (1931) for “*D. attemsii*” (Fig. 4).

The single relatively large sample in our hands, that from Osomba, shows the following variations in structure of metatergum 4. Most of the samples have rather long dorsal horns which often are even slightly curved caudad and set close to the caudal margin, with 2+2 subequal tubercles/cones in front. However, in one ♂ the situation is largely the same as described above for the ZSM ♂. It shows the gonopods typical of “*D. attemsii*” as clearly depicted by Attems (1931, 1938) (Fig. 5) and used for SEM here (Fig. 7), both horns are shorter, rather tuberculiform and clearly shifted forward off the caudal margin of the metatergum (the left horn also being nearly bifid), while the 1+1 central paramedian cones in front are a little higher than the lateral ones (Fig. 1A). All this is definitely evidence of the variability being purely individual.

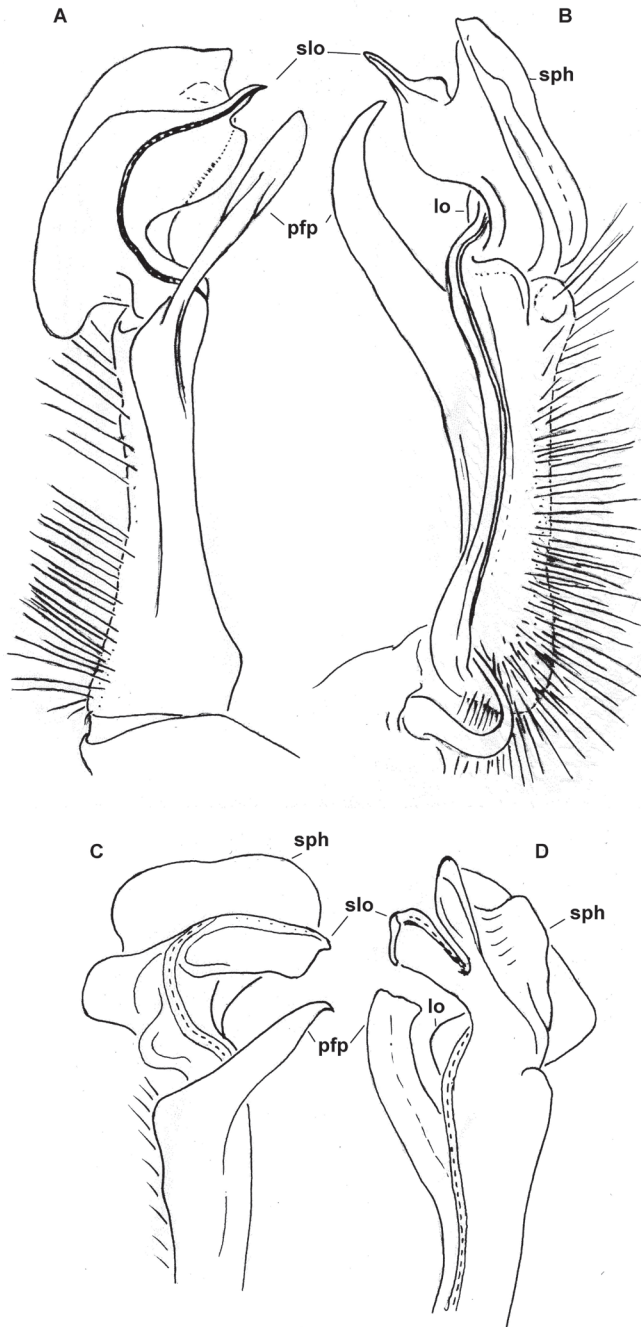


**Figure 1.** *Diaphorodesmus dorsicornis* (Porat, 1894). **A** Metatergum 4 of a ♂ (ZMUC) from Osomba/ Calabar, Nigeria, dorsal view **B** Anterior body part of a ♂ (NHMW) from Bibundi, Cameroon, dorso-lateral view **C, D** Anterior body part of a ♂ (MNHN) from Kumba, Cameroon, dorsolateral and dorsal views, respectively **E, F** Metaterga 4 and 5 of a ♂ (ZSM) from an unknown locality in Cameroon, dorso-lateral (4<sup>th</sup> to the left) and dorsal (4<sup>th</sup> at the bottom) views, respectively. Photos by J. Brecko (**A, C–F**) and N. Akkari (**B**).

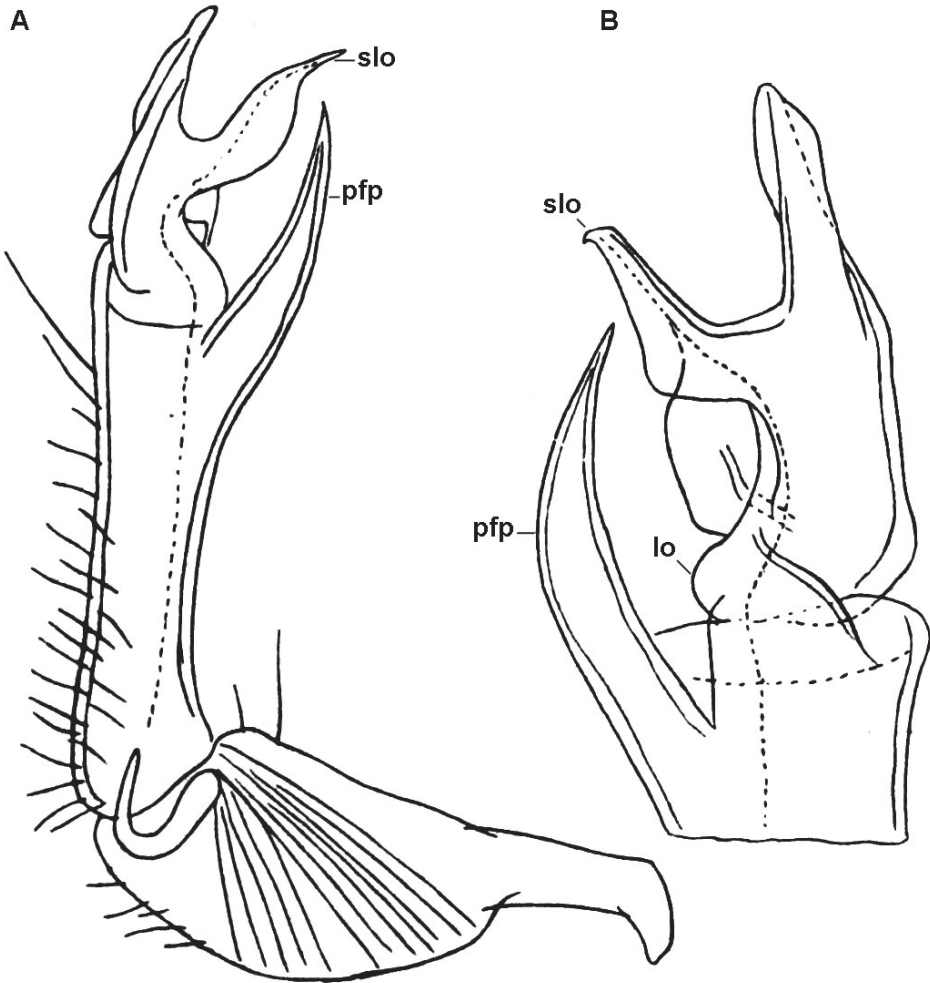


**Figure 2.** *Diaphorodesmus dorsicornis* (Porat, 1894), ♂ from Port Harcourt, Nigeria. **A, B** Anterior body part, sublateral and dorsal views, respectively. **C.** Metatergum 10, dorsal view. **D** Right gonopod *in situ*, ventral view **E, F** Left gonopod, mesal and lateral views, respectively. Del. R.L. Hoffman, drawn not to scale. Labels added by present authors; abbreviations explained in text.





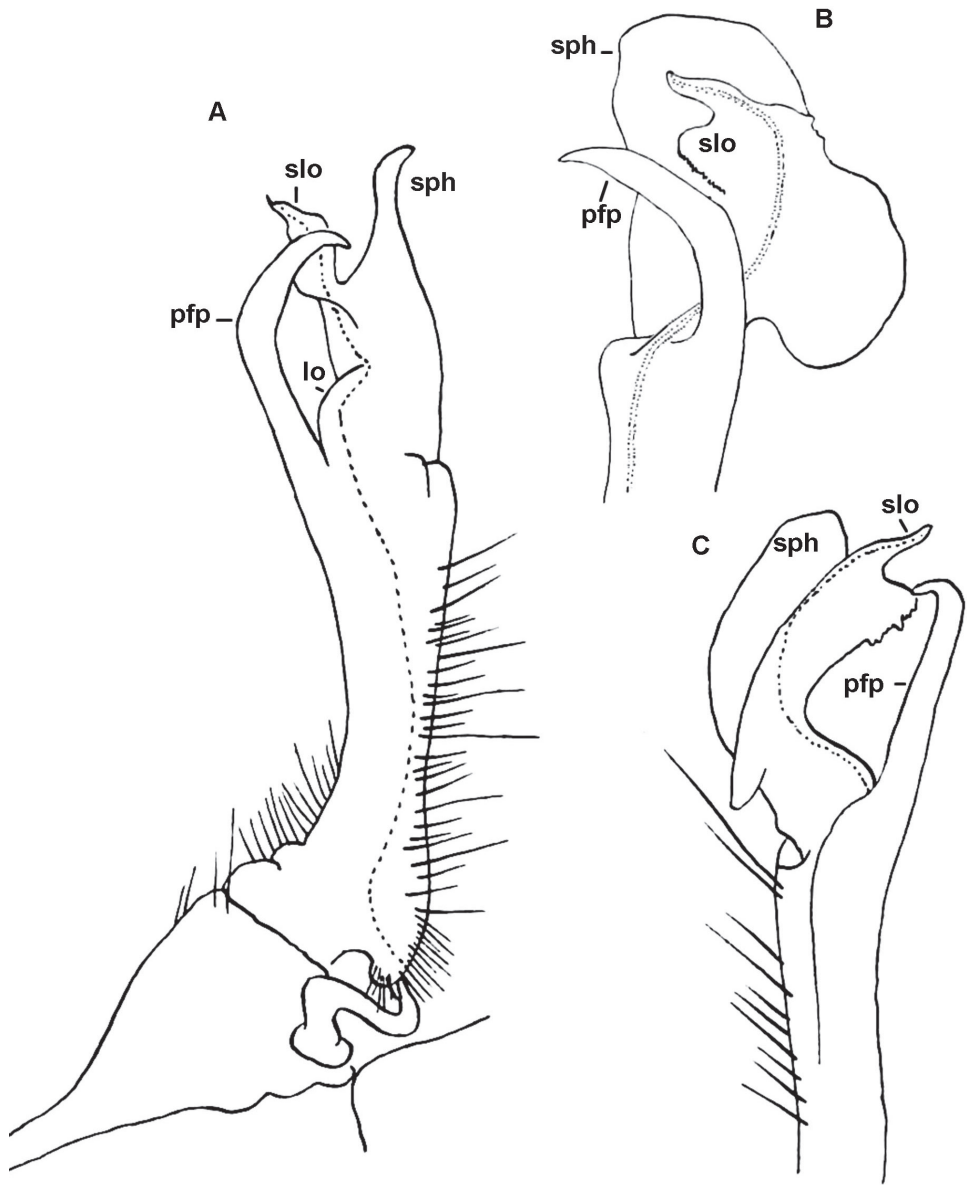
**Figure 3.** *Diaphorodesmus dorsicornis* (Porat, 1894). **A, B** Left gonopod of a ♂ syntype (NHRS) from an unspecified locality in Cameroon, lateral and mesal views, respectively **C, D** Distal part of the left gonopod of a syntype of “*D. attemsii* Verhoeff, 1938” (Hamburg Museum?) from the Botanical Garden in Victoria, Cameroon, lateral and mesal views, respectively. Del. R.L. Hoffman, drawn not to scale. Labels added by present authors; abbreviations explained in text.



**Figure 4.** *Diaphorodesmus dorsicornis* (Porat, 1894). Gonopods of a ♂ (ZSM) from an unspecified locality in Cameroon. **A** Right gonopod, mesal view **B** Tip of left gonopod, mesal view. Del. K.W. Verhoeff, drawn not to scale. After Verhoeff (1938). Labels added by present authors; abbreviations explained in text.

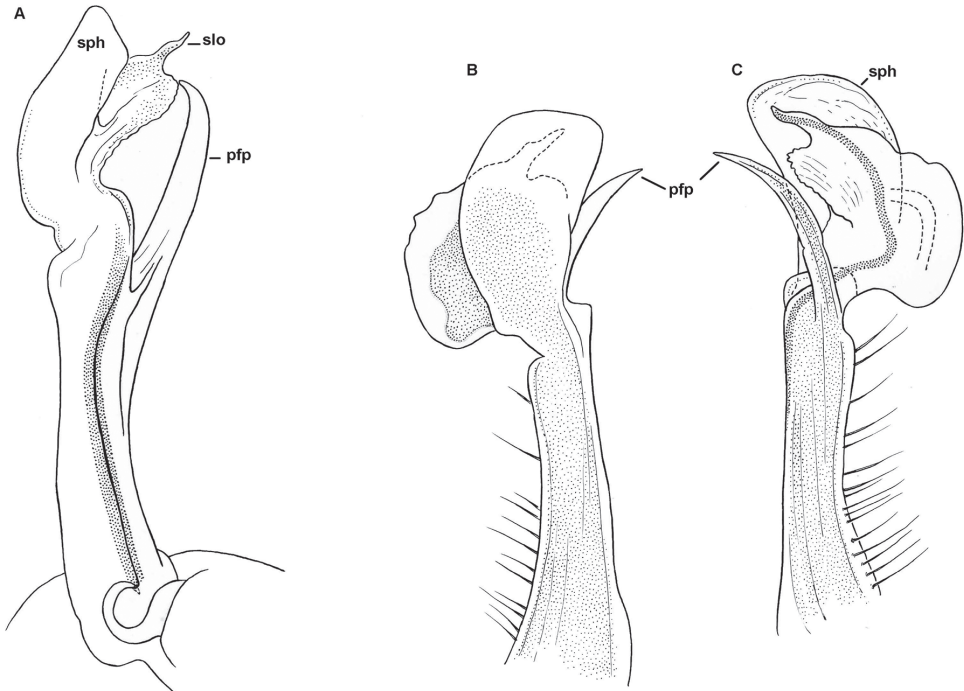
The NHMW series of “*D. attemsii*” syntypes, which contains 1 ♂ and 1 ♀ from Bibundi, 2 ♀♀ from Victoria, and a microscopic slide with the gonopods of a ♂ from Mukonje Farm, shows the same somatic variations as noted above (N. Akkari, in litt.). Thus, metatergum 4 of the ♂ from Bibundi (Fig. 1B) has typical horns, both rather high, slightly curved caudad and placed quite close to the posterior margin, whereas the cones in front are 2+2, the paramedian pair being slightly larger than the lateral one.

Hoffman, in his unpublished archives, provided the following distinctions between *D. dorsicornis* from *D. attemsii*, based solely on gonopod structure. The gonopod of “*D. attemsii*” was drawn from a ♂ taken at Victoria, southwestern Cameroon (apparently, the Hamburg Museum collection, see Weidner 1960).

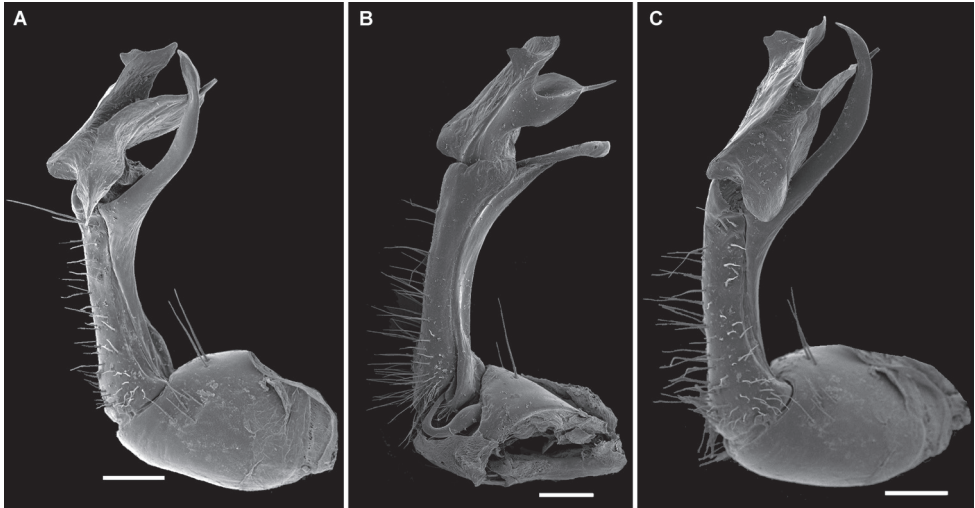


**Figure 5.** *Diaphorodesmus dorsicornis* (Porat, 1894). Gonopods of a ♂ syntype of “*D. attemsii* Verhoeff, 1938” (NHMW) from an unspecified locality in Cameroon. **A** Left gonopod, mesal view **B** Tip of right gonopod, anterior view **C** Most of telopodite of right gonopod, lateral view. Del. C. Attems, drawn not to scale. After Attems (1931). Labels added by present authors; abbreviations explained in text.





**Figure 6.** *Diaphorodesmus dorsicornis* (Porat, 1894). Gonopods of a ♂ (MNH) from Kumba, Cameroon. **A** Right gonopod, mesal view **B–C** Telopodite of right gonopod, ventral and anterior views, respectively. Del. N. Bertoncini (MNH). Labels added by present authors; abbreviations explained in text.



**Figure 7.** *Diaphorodesmus dorsicornis* (Porat, 1894). SEM micrographs of both gonopods of a ♂ of “*D. attemsii* Verhoeff, 1938” (ZMUC) from Osomba/Calabar, Nigeria. **A, C** Left gonopod, lateral and sub-lateral views, respectively **C** Right gonopod, mesal view. Scale bars: 0.2 mm.

<i>D. dorsicornis</i> Gonopod postfemoral process ( <b>pfp</b> ) long and slender, apically curved and pointed, expanded distally from a broad base; an inconspicuous rounded lobule ( <b>lo</b> ) between base of <b>pfp</b> and solenomere lobe ( <b>slo</b> ) (Fig. 3A, B).	<i>D. attemsii</i> Gonopod postfemoral process ( <b>pfp</b> ) relatively short, truncated apically, tapering regularly from a narrow base; a larger rounded lobe ( <b>lo</b> ) between base of <b>pfp</b> and solenomere lobe ( <b>slo</b> ) (Figs 3C, D & 5).
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Hoffman used Verhoeff's (1938) account of somatic differences (which actually do not hold, as the ZSM ♂ has the typical 2+2 cones in front of the dorsal horns!) to distinguish both *D. dorsicornis* and *D. attemsii* from what Hoffman evidently intended to describe as a new species. He also made several drawings of somatic and gonopodal characters, using a ♂ from Port Harcourt, southeastern Nigeria (Fig. 2). Its metatergum 4 may indeed show 3+3 cones in front of the horns (Fig. 2A), while its gonopod traits (Fig. 2D–F) match very closely those presented by Verhoeff (1938) for the ZSM ♂ (Fig. 4).

Comparing the gonopods of *Diaphorodesmus* samples from a number of often disparate localities across western Africa (see Porat 1894; Carl 1905; Attems 1931; Verhoeff 1938; VandenSpiegel et al. 2015, as well as our Figs 2D–F, 3–7), the variations observed in the relative sizes and shapes of **pfp**, **slo**, **lo** and **sph**, just like those of the above somatic features, seem to be random and too minor to consider more than individual. Therefore, we do not hesitate to formally synonymize *D. attemsii* Verhoeff, 1938 with *D. dorsicornis* (Porat, 1894), syn. n., treating the genus monospecific, albeit quite polymorphic. This conclusion is in accord with the vast distribution of *D. dorsicornis* in southeastern Nigeria, southwestern Cameroon and Equatorial Guinea, western Africa (Fig. 12).

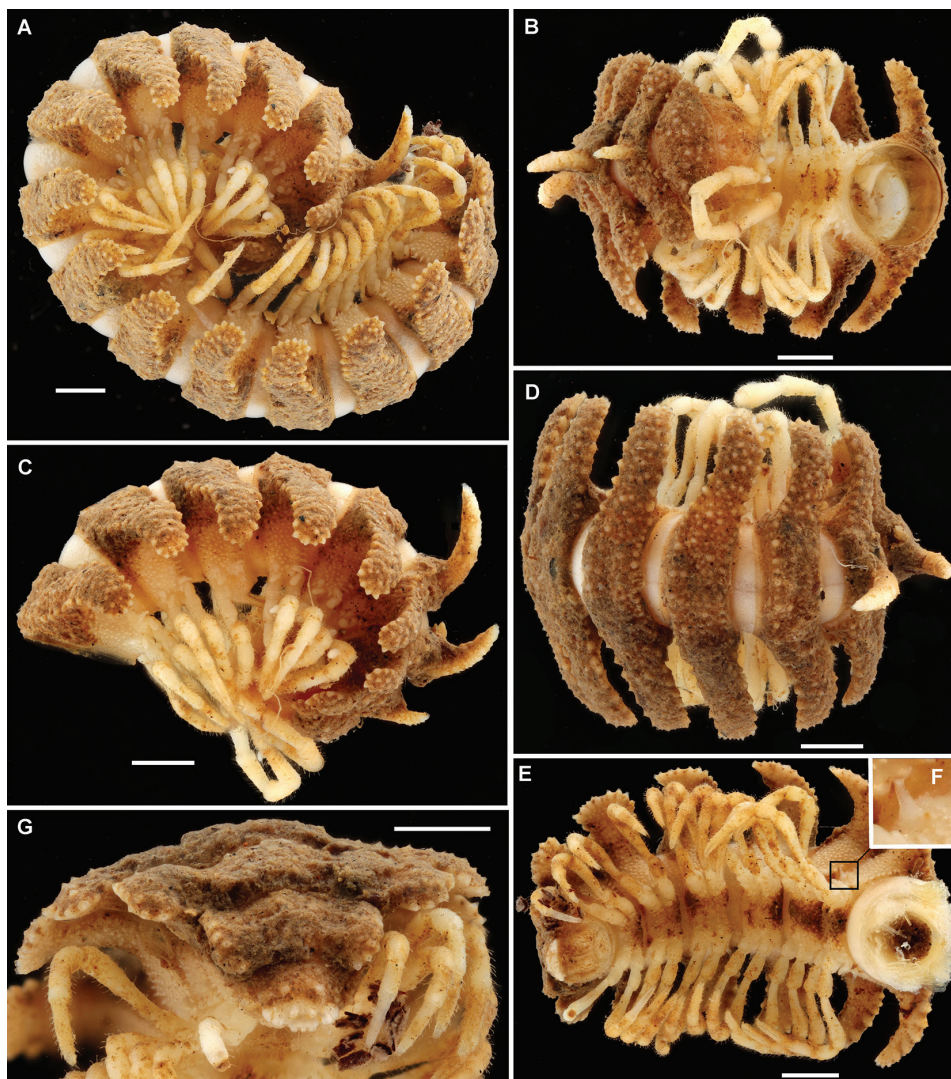
### *Diaphorodesmoides* gen. n.

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**Type species.** *Diaphorodesmoides lamottei* sp. n., by present designation.

**Name.** To emphasize the strong resemblance to *Diaphorodesmus* Silvestri, 1896, particularly in sharing the conspicuous dorsal horns on metaterga 2–4.

**Diagnosis.** A genus of Prepodesmidae, Chelodesmidae that differs by the presence of a single, conspicuous, increasingly long, dorsomedian horn on each of metaterga 2–4, coupled with the ozopores not being borne on porosteles, but opening flush dorso-laterally on the surface of poriferous paraterga; the spiracles tubiform, unusually long and slender; and the gonopod telopodites being suberect, *in situ* directed forward, held parallel to each other, not crossing mesally; prefemoral (= densely setose) part erect, taking up ca 2/3 of total gonotelopodite length, without femorite, but with a more complex dorsal postfemoral process (**pfp**), set off from acropodite by a distinct cingulum; acropodite clearly twisted, divided parabasally into three large lobes, the middle of which forming a large solenomere lobe (**slo**) with only a minor solenomere proper (**sl**) on top, **slo** being neatly squeezed between a larger mesal uncus (**u**) and a smaller lateral branch (**lb**), both **u** and **lb** forming a solenophore.



**Figure 8.** *Diaphorodesmoides lamottei* sp. n., ♂ holotype. **A** Habitus, lateral view **B–D** Anterior part of body, ventral, lateral and dorsal views, respectively **E** Caudal part of body, ventral view **F** Spiracle, subventral view **G** Last few body segments, caudal view. Scale bars: 1.0 mm (**A–E**, **G**), not to scale (**F**). Photos by J. Brecko.

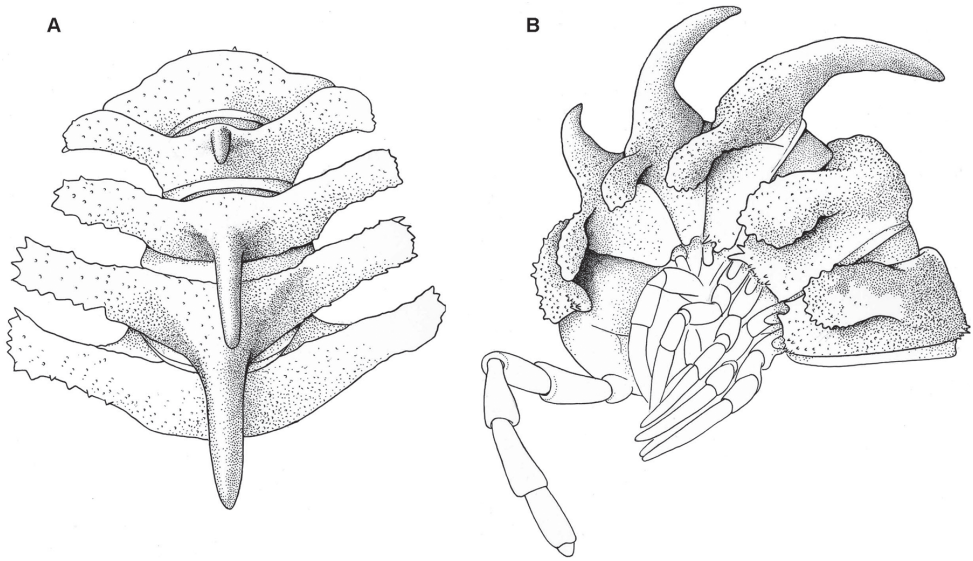
***Diaphorodesmoides lamottei* sp. n.**

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Figs 8–12

**Name.** To honour Maxime Lamotte, the collector.

**Material examined. Holotype.** CAMEROON: ♂ (MNHN JB253), KumbaEtam, 25.XI.1975, leg. M. Lamotte.



**Figure 9.** *Diaphorodesmoides lamottei* sp. n., ♂ paratype. **A, B** Anterior part of body, dorsal and lateral views, respectively. Del. N. Bertoncini (MHNH), drawn not to scale.

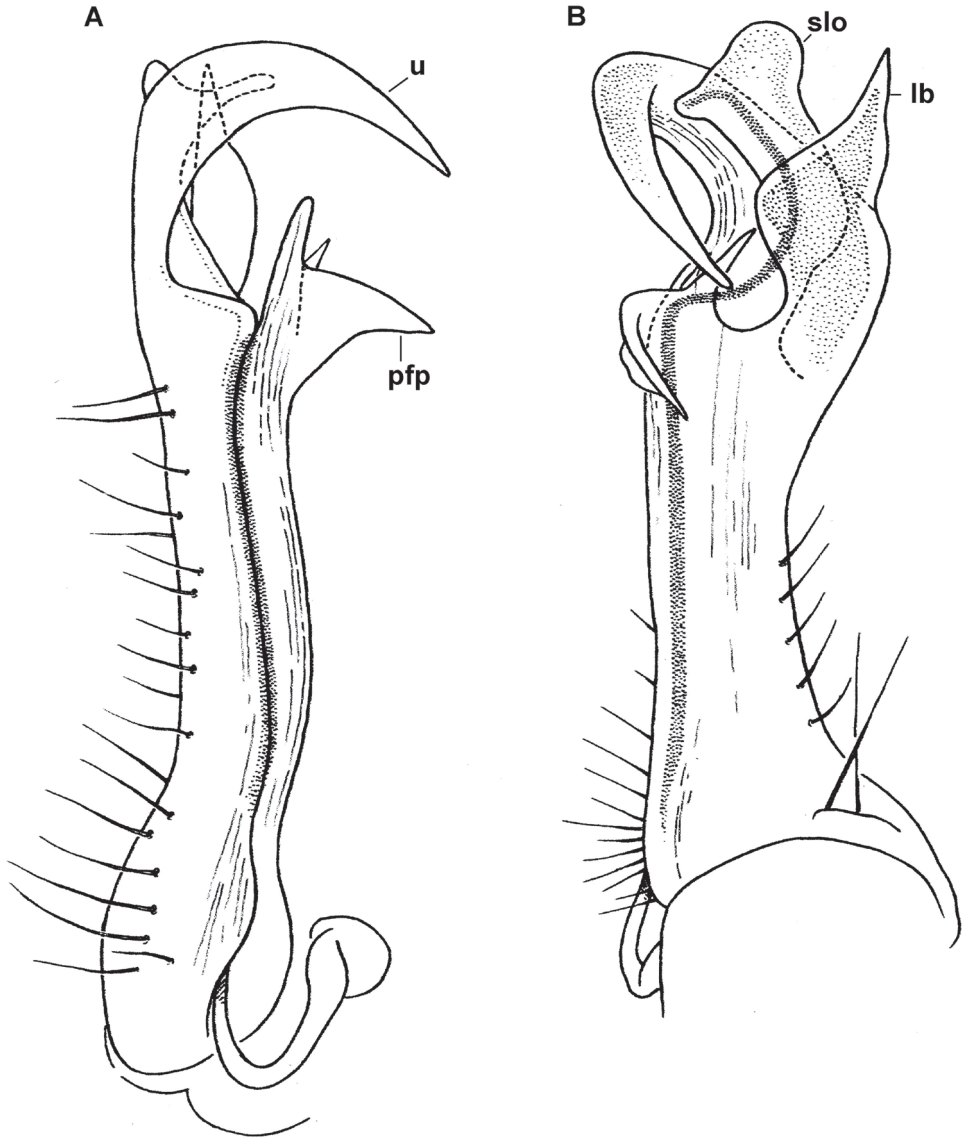
**Paratype.** CAMEROON: 1 ♂ (MHNH JB253), same place, together with holotype.

**Description.** LENGTH of holotype ca 26 mm, width of midbody pro- and metazonae 2.0 and 5.7 mm, respectively. The sole ♂ paratype is ca 27 mm long, 2.1 and 5.8 mm wide on pro- and metazonae, respectively. Metaterga and epiproct dirty brown dorsally, with lighter granulations and tubercles (Fig. 8); head and ventral sides of paraterga a little lighter, brownish; antennae, sides, venter and legs light, yellowish.

Head densely granulate-microtuberculate and setose on dorsal face, interantennal isthmus about half as broad as diameter of antennal socket. Antennae long and only slightly clavate, *in situ* reaching behind body segment 3 when stretched dorsally; antennomeres 5 and 6 each with a dorso-apical group of tiny bacilliform sensilla; in length, antennomere  $6 > 2 = 5 > 1 > 7$ ; apical segment with usual four sensory cones.

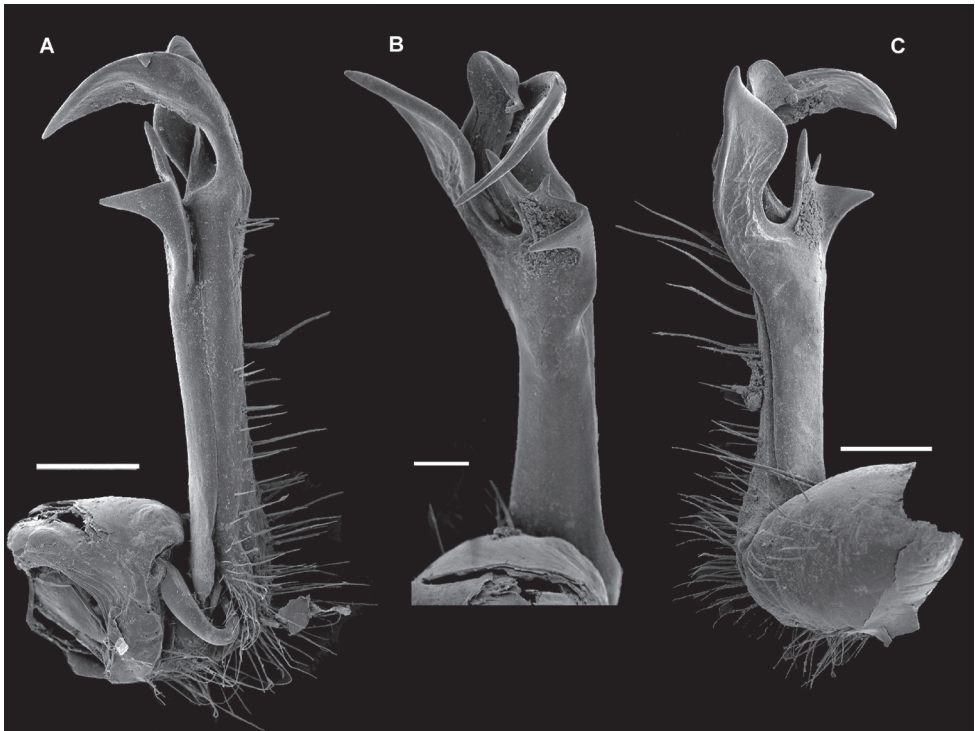
Body with 20 segments (♂). In width, segment head < collum < segment 2 < 3 < 4 < 5 < 6 = 15; body rapidly tapering from segment 18 towards telson. Collum transversely ellipsoid, not covering the head from above; sides narrowly rounded; dorsal surface densely irregularly granulate-tuberculate (Figs 8C, 9B). Dorsum strongly and mostly regularly convex (Figs 8, 9). Only prozonae smooth and shining; metazonae dull, densely tuberculate-granulate all over, devoid of a ceratogenous crust, but in places clothed with a crust of earth dirt; dorsal surface of metaterga and ventral sides of paraterga with 6–8 irregular transverse rows of small grains, tubercles or short spines, only marginal rows being regular and, on paraterga, composed of ca 10 tubercles in each fore and caudal row, and of 5–6 at lateral edge; stricture smooth. Metaterga 2–4 each with an increasingly prominent, caudally curved and nearly sharp, microgranulate, subcylindrical, central horn (Figs 8A–D, 9). Metaterga 2–5 each with a small, but





**Figure 10.** *Diaphorodesmoides lamottei* sp. n., ♂ paratype. **A, B** Right gonopod, mesal and sublateral views, respectively. Del. N. Bertoncini (MHNH), drawn not to scale.

evident impression at base of paraterga, following paraterga (nearly) regularly convex, continuing the convex outline of mid-dorsal region. Paraterga very broad, set at about upper 1/3 of body, tips regularly rounded, mostly lying at about half of body height and slightly bent down; only paraterga 16–19 increasingly clearly drawn behind rear tergal margin, 19<sup>th</sup> sharp. Sides below paraterga densely granulate, grains in caudal row being longer, spiniform and sharp. Ozopores barely visible, open flush on surface

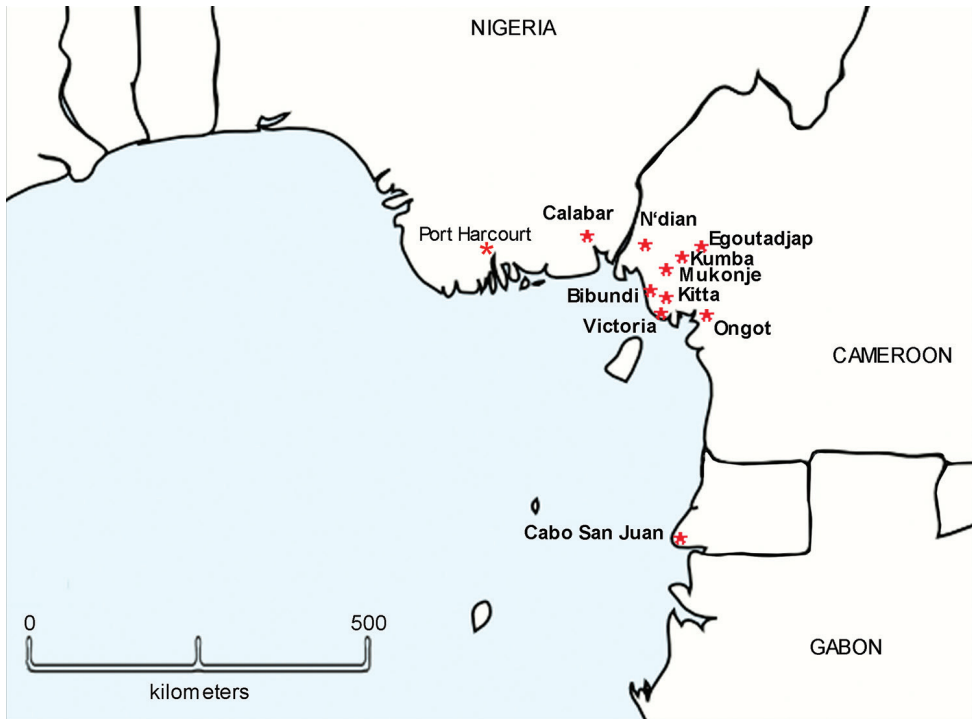


**Figure 11.** *Diaphorodesmoides lamottei* sp. n., ♂ holotype. **A–C** SEM micrographs of left gonopod, mesal, anterior and lateral views, respectively. Scale bars: 0.2 mm.

near midlength slightly above lateral edge of paraterga; pore formula untraceable. A thin, dark, axial line sometimes traceable through a transparent tegument, best visible on collum and prozonae. Pleurosternal carinae wanting. Limbus entire, translucent. Epiproct short, small, spade-shaped, strongly flattened dorsoventrally, subtruncate, dorsally granulate-tuberculate (Fig. 8G). Hypoproct densely granulate-tuberculate, roundly subtrapeziform, with 1+1 caudal setae very distinctly separated and borne on minute knobs (Fig. 8E). Paraprocts likewise densely granulate-tuberculate (Fig. 8E).

Sterna broad, nearly twice as broad as coxa length, almost flat, densely setose (Fig. 8E). Gonapophyses on ♂ coxae 2 vestigial. Spiracles (Fig. 8A, C, F) tubiform, remarkably long and slender. Legs very long, about 2.0 times as long as midbody height (♂), very slender; in length, femur > tarsus > tibia > prefemur = postfemur = coxa; claw very small, very slightly curved; ventral surface of tarsi densely setose, but forming no brushes.

Gonopod aperture transversely ovoid, large, its lateral and posterior edges slightly elevated, fully concealing gonocoxae and bases of telopodites. Gonopods relatively complex (Figs 10, 11). Coxites medium-sized, subcylindrical, fused at base to a small membranous sternal remnant, poorly setose distodorsally, including a pair of very closely placed, distalmost and particularly long setae. Cannulae slender, without peculiarities. Telopodites *in situ* directed forward, held subparallel to each other, suberect, not crossing each other mesally. Prefemoral (= densely setose) part erect, taking up ca



**Figure 12.** Distributions of *Diaphorodesmus dorsicornis* (Porat, 1894) (only known localities, arranged more or less from northwest to south; SE Nigeria: Port Harcourt; Osomba 56 mi from Calabar; SW Cameroon: N'dian, Egoutadjap, Kumba, Mukonje, Bibundi, Kitta, Victoria, Ongot; Equatorial Guinea: Cabo San Juan) and *Diaphorodesmoides lamottei* sp. n. (only Kumba).

2/3 of total gonotelopodite length, without femorite, but with a relatively short, complex, tridentate, dorsal postfemoral process (**pdfp**), set off from acropodite by a distinct cingulum; acropodite clearly twisted, divided parabasally into three large lobes, the middle of which forming a large solenomere lobe (**slo**) with only an indistinct, small solenomere proper on top, **slo** being neatly squeezed between a larger mesal uncus (**u**) and a smaller, subtriangular, lateral branch (**lb**), both **u** and **lb** forming a solenophore.

**Remark.** At least at Kumba, the above new genus and species seems to occur sympatrically with *Diaphorodesmus dorsicornis* (Fig. 12). The label reading “KumbaEtam” is somewhat dubious. ‘Etam’ is a locality about 15 km NE of Kumba in Cameroon. The locality may therefore mean ‘between Kumba and Etam’ or ‘in the Kumba-Etam area’.

## Acknowledgements

Henrik Enghoff (ZMUC), Roland Melzer and Jörg Spelda (both latter from the ZSM) kindly rendered us for study certain material under their care. Nesrine Akkari (NHMW) most helpfully provided all necessary information concerning the type se-



ries of one of the revised species. The second author is greatly obliged to the Musée Royal de l'Afrique Centrale, Tervuren, Belgium for the invitation to join this project. Special thanks go to Rowland M. Shelley (Raleigh, NC, U.S.A.) for the provision of the unpublished relevant part of the late Richard L. Hoffman's archives housed at the Virginia Museum of Natural History, Martinsville, Virginia, U.S.A., to Jonathan Brecko (MRAC) for taking the colour pictures and to Christophe Allard (MRAC) for technical assistance. We thank cordially also the administration of the Virginia Museum of Natural History for their cooperation.

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# New species and records of Uropodina mites from Iran (Acari, Mesostigmata)

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## Abstract

In this paper, a new species of the genus *Nenteria* Oudemans, 1915 is described on the basis of adult female and male specimens collected in soil and litter in parks in Kerman, southeastern Iran, and Tehran, northern Iran. A key to the Iranian species of *Nenteria* is also presented, and *Trachycilliba abantica* (Bal & Özkan, 2007) is reported for the first time in Iran.

## Keywords

Trematuridae, soil-dwelling mites, Uropodidae, Uropodina

## Introduction

Mites of the infraorder Uropodina occur in forest soil and leaf litter, moss, rotting wood, dung, beach wrack, as well as in the nest of mammals, birds, and insect (Karg 1989, Wiśniewski and Hirschmann 1993, Błoszyk 1999, Maśán 2001, Lindquist et al. 2009). The classification of Uropodina, especially at higher level is not yet stable. For instance, the group was divided into five superfamilies (Protodinychoidea, Polyaspidoidea, Thinozerconoidea, Uropodoidea, Diarthrophalloidea) and 13 families by Lindquist et al. (2009), and into four superfamilies (Microgynioidea, Thinozerconoidea, Uropodoidea, Diarthrophalloidea) and 35 families by Beaulieu et al. (2011).

Halliday (2016) catalogued the genera of Uropodina based on the classification of Beaulieu et al. (2011) and listed 300 genus-group names and their type species.

The Uropodoidea or “higher uropodines” (Evans 1972) is the largest superfamily of Uropodina and comprises over 2200 described species (Wiśniewski and Hirschmann 1993, Beaulieu et al. 2011). Although several attempts have been done to clarify the classification of uropodine mites, the boundaries of several groups of these mites, including the families and genera are not clear yet, such as the genus *Nenteria* that was erected by Oudemans (1915) with the type species of *Uropoda tropica* Oudemans, 1905. Some authors considered *Nenteria* as a genus of Uropodidae (e.g. Krantz and Aniscough 1990, Kadite and Petrova 1977), or placed it in the family Trematuridae (e.g. Karg 1989, Mařán 2001), or in the family Nenteriidae (e.g. Hirschmann 1979, Hirschmann and Wiśniewski 1985, Farrier and Hennessey 1993, Beaulieu et al. 2011, Kotschán 2012, 2014). Hirschmann and Wiśniewski (1985) stated that there were 109 described species of the genus *Nenteria* and divided them into eight species group, and later, in 1993, they mentioned 124 species in the genus (Wiśniewski and Hirschmann 1993).

Iranian mites of Uropodoidea (*sensu* Beaulieu et al., 2011) are poorly known. Until now, only 34 species of this superfamily have been reported in Iran, including three species of the genus *Nenteria* (*N. breviunguiculata* Willmann, 1949; *N. stammeri* Hirschmann & Z.-Nicol, 1962; *N. stylifera* Berlese, 1904) and two species of *Trachycilliba* Berlese, 1903 (reported as members of *Neodiscopoma* Vitzthum, 1943: *N. splendida* (Kramer, 1882) and *N. persica* Kazemi & Kotschán, 2007) (Kazemi and Rajaei 2013, Arjomandi et al. 2013, Arjomandi and Kazemi 2014, in press, Kazemi et al. 2014, Kazemi and Kotschán 2014). The purpose of this paper is to describe a new species of *Nenteria* from Iran, present a key to the species of *Nenteria* occurring in the country, and report a species of *Trachycilliba* that is previously unknown in Iran.

## Material and methods

The mite specimens were extracted from soil of parks in Kerman and Tehran cities by Berlese-Tullgren funnels, cleared in Nesbitt’s fluid and then mounted in Hoyer’s medium on microscope slides.

Morphological observations, measurements and illustrations were made using a compound microscope equipped with differential interference contrast and phase contrast optical systems (Olympus BX51). Measurements are given in micrometers ( $\mu\text{m}$ ). Dorsal shield length and width were respectively taken from the anterior to posterior shield margins along the midline and from the lateral margins at the broadest level. The length and width of the epigynal shield were measured from the anterior to posterior margins of the shield along the midline, and from the lateral margins of the shield at the broadest point, respectively. The length of the second cheliceral segment was measured from the base to the apex of the fixed digit, and its width at the broadest point. The length of the fixed cheliceral digit was taken from anterior level of nodus to the apex, and that of the movable digit from the base to apex. The legs length was

taken from the base of the coxa to the apex of the tarsus, excluding the ambulacrum. Notation for setae on ventral and dorsal idiosoma mostly follows those of Lindquist and Evans (1965).

## Taxonomy

### Family Trematuridae

**Note.** The genus *Nenteria* was placed in the family Nenteriidae by Hirschmann (1979), but he never published any diagnosis or description for the family. Although several subsequent authors referred to this family name (such as those mentioned in introduction), the family has never been described, and Nenteriidae remains a *nomen nudum* (Halliday, 2016). We therefore place *Nenteria* in the family Trematuridae, following Karg (1989) and Mařán (2001).

### Genus *Nenteria* Oudemans, 1915

**Type species.** *Uropoda tropica* Oudemans, 1905

**Diagnosis.** The genus diagnosis of Mařán (2001) was followed.

#### *Nenteria bastanii* sp. n.

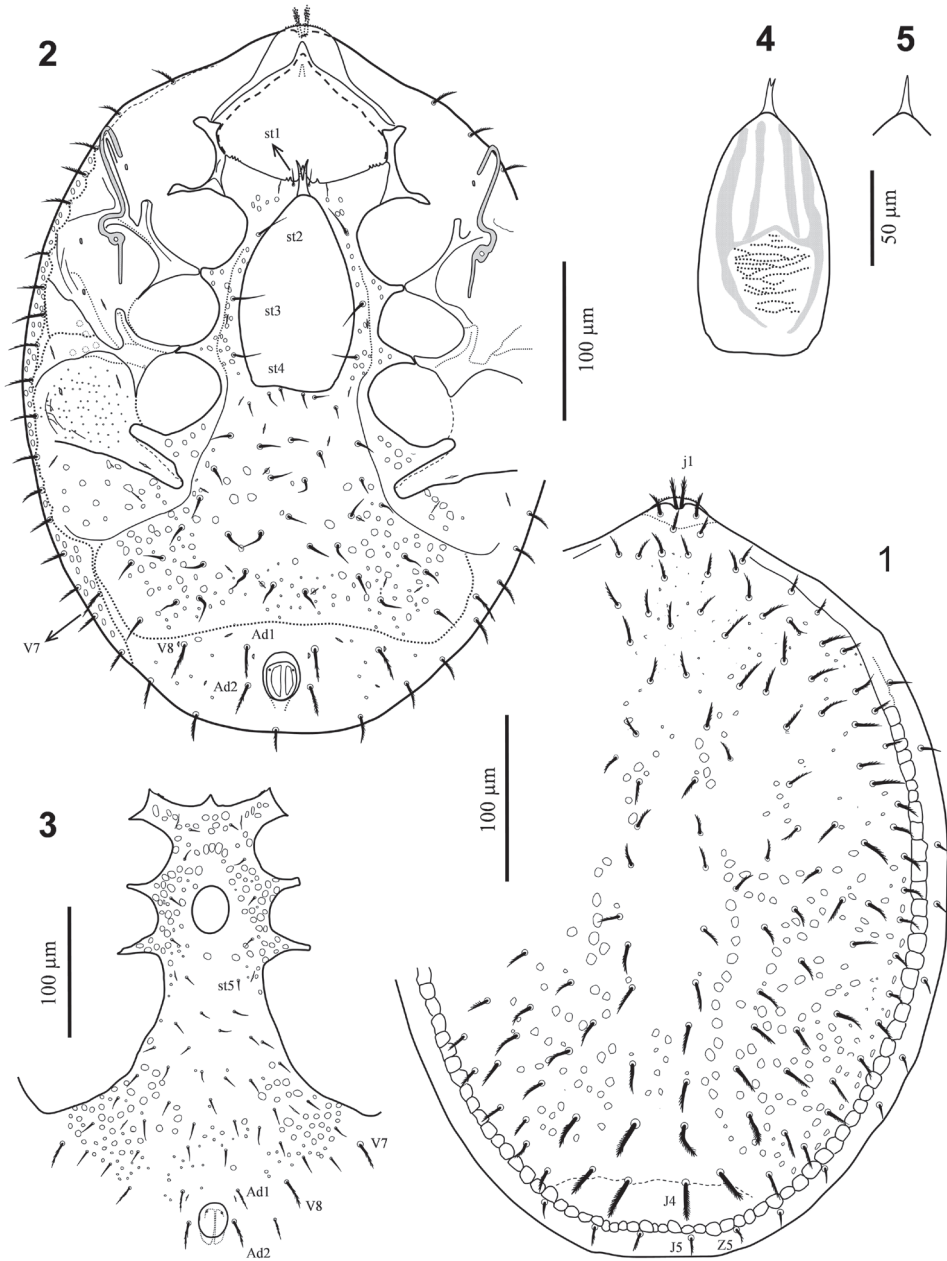
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Figures 1–16

**Diagnosis (adult female and male).** Dorsal shield with 75 pairs of short and pectinate setae, and 2–4 unpaired median setae on propodosomal region. Marginal setae short and smooth, except pectinate setae *J*5 and *Z*5. Female epigynal shield iron-shape, posteriorly reaching to mid-level of coxae IV, with an apical anterior spike, occasionally bifid at tip; shield surface smooth. Sternal setae *st*1–5 smooth. Female ventral region behind epigynal shield bears 22–23 pairs of setae, including *Ad*1–2 and *st*5, and with 22 pairs in male, excluding *st*4–5, setae mostly short and acicular, except *Ad*1–2, *V*7–8 longer and pectinate. Peritremes with a hook-like anterior extensions and without anterior projections to forward. Anterior edge of epistome bifid, without median hyaline flap. Cheliceral movable digit with a median tooth. Claws of leg I well-developed, sub-equal in size to other leg claws. Dorsal setae in femur, genu and tibia I of male mostly thicker than those in female.

**Description. Female** (n = 4). Idiosoma oval-shape, brown in color, 461–494 µm long, 336–346 µm wide.

**Dorsal idiosoma** (Fig. 1). Dorsal shield surface ornamented with sparse sub-circular pits, more densely on opisthonotal region, median region of shield smooth. Dorsal



**Figures 1–5.** *Nenteria bastanii* sp. n. **1** Female, dorsal idiosoma **2** Female, ventral idiosoma **3** Male, holovenral region of idiosoma **4** Female, epigynal shield **5** Female, anterior spike of epigynal shield.

shield setae 12–30  $\mu\text{m}$  long, mostly slightly pectinate, *j1* (23–24  $\mu\text{m}$ ) apically plumose, posterior setae slightly thicker, longer and densely pectinate, *j4* longest (27–30  $\mu\text{m}$ ). Marginal shield narrow, bearing nine pairs of smooth and two pairs (*j5*, *z5*) of

pectinate setae, 12–16  $\mu\text{m}$  long. With 20 pairs of submarginal plus one postanal seta, 18–23  $\mu\text{m}$  long, situated subventrally.

*Ventral idiosoma* (Figs 2, 4–5, 9). Tritosternal base narrow, cylindrical, 28–31  $\mu\text{m}$  long, 7–9  $\mu\text{m}$  wide, with a pair of small denticles lateromedially and a pair apically; laciniae serrate, 26–31  $\mu\text{m}$  long, apically trifid. Anterior margin of sternal region of holovenral shield with a median projection flanked by 1–2 small teeth; shield surface ornamented with sub-circular pits; sternal setae smooth, *st1* 10–11  $\mu\text{m}$  long, adjacent, inserted near anterior margin of shield, *st2–4* 16–21  $\mu\text{m}$  long, subequal. Epigynal shield iron-shape, 125–136  $\mu\text{m}$  long (excluding anterior spike), 73–84  $\mu\text{m}$  wide, with a relatively long anterior spike (18–20  $\mu\text{m}$ ), occasionally bifid at apex, shield surface smooth, posterior margin of shield truncate, ending at mid-level of coxae IV. Ventrianal region of holovenral shield with 22–23 pairs of setae, including *st5*, mostly smooth (12–18  $\mu\text{m}$ ), except pectinate para-anal setae *Ad1* (24–26  $\mu\text{m}$ ), *Ad2* (22–24  $\mu\text{m}$ ) and ventral setae *V7* (19–22  $\mu\text{m}$ ), *V8* (21–24  $\mu\text{m}$ ). Peritremes without anterior angle of 90°, poststigmatic section 22–26  $\mu\text{m}$  long. Anal opening oval-shape, 34–37  $\mu\text{m}$  long, 24–28  $\mu\text{m}$  wide. Surface between pedofossae III–IV with fine reticulate pattern.

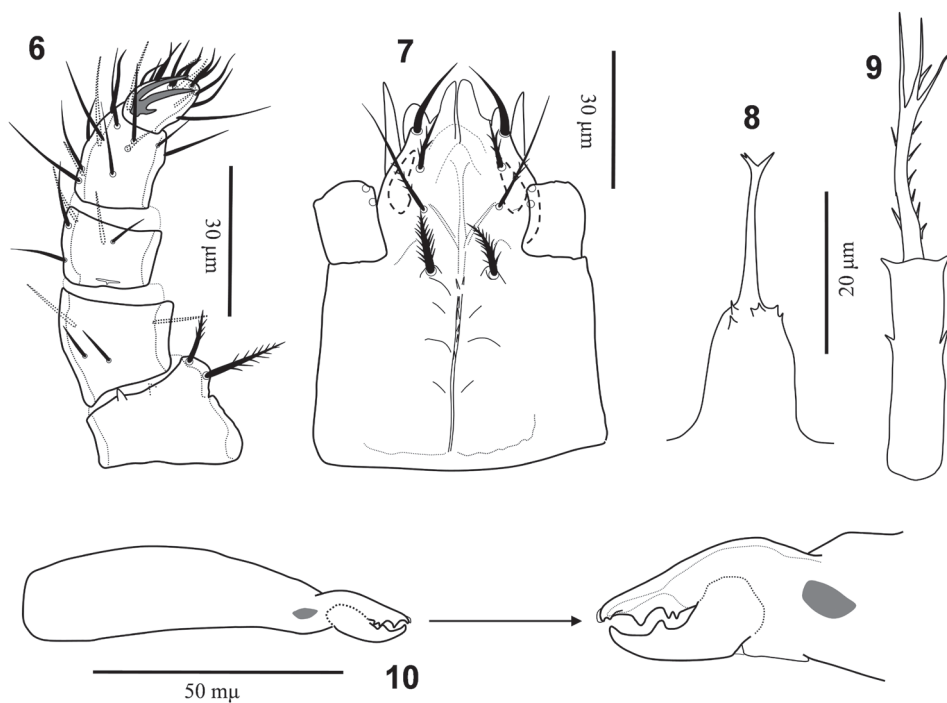
*Gnathosoma* (Figs 6–7, 9–10). Base of epistome columnar and relatively wide, bearing some small denticulate outgrowths subapically, with a median narrow elongate projection, apically bifid (Fig. 8). Corniculi horn-like (14  $\mu\text{m}$ ), ventrally covered by subcapitulum; internal malae and paralaciniae smooth (Fig. 7). Hypostomal setae *h1* (16–18  $\mu\text{m}$ ) smooth, thickened, inserted on small protuberances, *h2* (11–15  $\mu\text{m}$ ) serrate, *h3* (26–28  $\mu\text{m}$ ) elongate, slender, with some barbs in basal half, capitular setae *h4* (14–16  $\mu\text{m}$ ) thickened, plumose. Deutosternal groove narrow, with few denticles behind capitular setae. Second segment of chelicera 81–83  $\mu\text{m}$  long, 18–19  $\mu\text{m}$  wide; fixed cheliceral digit 19–21  $\mu\text{m}$  long, with a minute sub-apical denticle, and 2–3 teeth; movable digit 15–17  $\mu\text{m}$  long, with a median tooth. Palp 74–80  $\mu\text{m}$  long; palp chaetotaxy as “*Uropoda*-type” (*sensu* Evans, 1963): trochanter 2, femur 4, genu 5, tibia 14, tarsus 15; all setae smooth, except *v1–2* in trochanter pectinate.

*Legs* (Figs 11–14, 16). Leg chaetotaxy “*Uropoda*-type” (*sensu* Evans, 1972). All legs with claws; leg I with a long sub-terminal seta (49–54  $\mu\text{m}$ ). Chitinous membrane present in coxae I, trochanter I–II, femura I–IV. Lengths of legs I–IV 242–256  $\mu\text{m}$ , 185–198  $\mu\text{m}$ , 192–214  $\mu\text{m}$  and 245–247  $\mu\text{m}$ , respectively. Lengths of femora I–IV 49–55  $\mu\text{m}$ , 53–61  $\mu\text{m}$ , 48–55  $\mu\text{m}$ , 56–62  $\mu\text{m}$ ; genua I–IV 18–21  $\mu\text{m}$ , 21–23  $\mu\text{m}$ , 19–22  $\mu\text{m}$ , 20–24  $\mu\text{m}$ ; tibiae I–IV 17–21  $\mu\text{m}$ , 25–26  $\mu\text{m}$ , 21–24  $\mu\text{m}$ , 23–27  $\mu\text{m}$ ; tarsi I–IV 56–62  $\mu\text{m}$ , 53–56  $\mu\text{m}$ , 56–59  $\mu\text{m}$ , 78–81  $\mu\text{m}$ , respectively. Claws on tarsus I subequal to other legs claws (Fig. 16). Pretarsi I–IV 10–12  $\mu\text{m}$ , 18–20  $\mu\text{m}$ , 17–19  $\mu\text{m}$ , 22–25  $\mu\text{m}$ , respectively. Leg setae mostly narrow, needle-like and short, as figures 11–14.

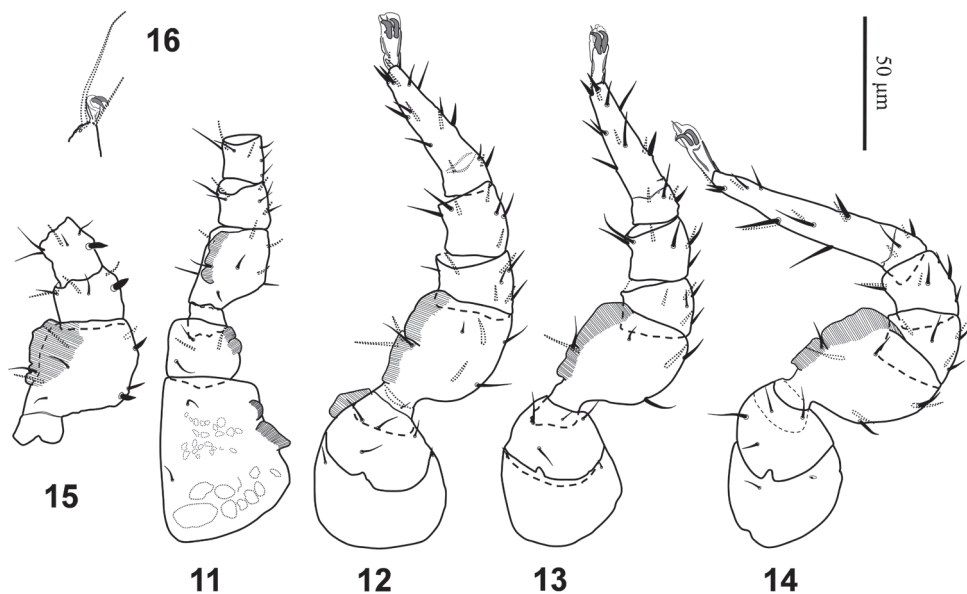
**Male** (*n* = 3). Idiosoma 440–464  $\mu\text{m}$  long, 343–357  $\mu\text{m}$  wide.

*Dorsal shield*. Dorsal shield characters similar to those in female. Dorsal shield setae 9–28 long, *j1* 22–24  $\mu\text{m}$ , *J4* 24–28  $\mu\text{m}$  long. Marginal setae 9–14  $\mu\text{m}$  long; submarginal and postanal setae 17–22  $\mu\text{m}$  long.

*Ventral shield*. Sternal setae smooth, *st1* 10–12  $\mu\text{m}$ , *st2* 17–19  $\mu\text{m}$ , *st3* 13–17  $\mu\text{m}$ , *st4–5* 12–15  $\mu\text{m}$  long. Operculum oval-shape, 38–42  $\mu\text{m}$  long, 25–33  $\mu\text{m}$  wide. Ven-



**Figures 6–10.** *Nenteria bastanii* sp. n. Female, **6** Palp **7** Subcapitulum **8** Epistome **9** Tritosternum **10** Chelicera.



**Figures 11–16.** *Nenteria bastanii* sp. n. **11–14** Female, legs I–IV, posterolateral view **15** Male, leg I: femur-tibia **16** Female, apical region of tarsus I and pretarsus.



trianal region with 20 pairs of simple, smooth setae (12–15  $\mu\text{m}$ ), except pectinate setae *Ad1*–2 (22–25  $\mu\text{m}$ ), *V7*–8 (20–23  $\mu\text{m}$ ).

**Gnathosoma.** Hypostomal seta shape similar to those in female, *h1* 14–16  $\mu\text{m}$ , *h2* 12–15  $\mu\text{m}$ , *h3* 29–31  $\mu\text{m}$ , *h4* 14  $\mu\text{m}$  long. Corniculi horn-like (14  $\mu\text{m}$ ). Fixed and movable digit of chelicerae similar to female, fixed digit 19–20  $\mu\text{m}$  long, movable digit 15–16  $\mu\text{m}$  long. Palp 73  $\mu\text{m}$  long.

**Legs** (Fig. 15). Leg chaetotaxy and chitinous membrane similar to those in female, but dorsal setae in femur I, genu I and tibia I mostly thickened in male. Lengths of legs I–IV 232–273  $\mu\text{m}$ , 213–248  $\mu\text{m}$ , 200–212  $\mu\text{m}$ , 236–251  $\mu\text{m}$ , respectively. Lengths of femora I–IV 44–51  $\mu\text{m}$ , 57–61  $\mu\text{m}$ , 56–60  $\mu\text{m}$ , 56–62  $\mu\text{m}$ ; genera I–IV 19–20  $\mu\text{m}$ , 20–25  $\mu\text{m}$ , 21–25  $\mu\text{m}$ , 23–28  $\mu\text{m}$ ; tibiae I–IV 18–20  $\mu\text{m}$ , 20–25  $\mu\text{m}$ , 21–25  $\mu\text{m}$ , 24–27  $\mu\text{m}$ ; tarsi I–IV 55–59  $\mu\text{m}$ , 52–56  $\mu\text{m}$ , 55–59  $\mu\text{m}$ , 82–87  $\mu\text{m}$ , respectively. Pretarsus I 9–12  $\mu\text{m}$  long, pretarsi II–IV 18–22  $\mu\text{m}$  long. Apical long seta on tarsus I 51–56  $\mu\text{m}$  long.

**Material examined.** Holotype: female, southeastern Iran, Kerman Province, Kerman, Shora Park (30°29'84"N; 57°07'10"E), 1761 m a.s.l., from soil, 28 Sept 2015, coll. S. Kazemi, deposited in Acarological Collection, Institute of Science and High Technology and Environmental Sciences, Graduate University of Advanced Technology, Kerman, Iran (ACISTE). Paratypes: four females and two males with same collection data, deposited in ACISTE; three females and one male, Kerman, Pardis Park (30°29'81"N; 57°07'01"E), 1760 m a.s.l., from soil, 16 Aug 2015, coll. S. Abolghasemi, deposited in ACISTE; two females and one male collected in Tehran Province, Tehran, Taleghani Park (35°45'13"N; 51°25'26"E), 1424 m a.s.l., from soil and litter, 15 Sept 2015, coll. S. Kazemi, deposited in ACISTE.

**Etymology.** The species is named in honor of the famous current Iranian historian, poet, translator and writer who died in March 2014, Prof. Mohammad Bastani Parizi.

**Remarks.** The new species can be easily distinguished from other described species of the genus by presence of 22–23 pairs of setae in ventral region of the holoventral shield behind the epigynal plate (including setae *st5*) in female and 22 pairs in male (excluding *st4*–5).

### Key to the known Iranian species of *Nenteria* (female)

- 1 Peritreme with an anterior projection, curved to the front in a 90° angle ..... *N. stylifera* Berlese, 1904
- Peritremes without anterior projections to forward ..... 2
- 2 Opisthogastric region behind epigynal shield with 22–23 pairs of setae; epigynal shield with an apical narrow spike ..... *N. bastanii* sp. n.
- Opisthogastric region behind epigynal shield with 9–10 pairs of setae; epigynal shield without narrow apical spike ..... 3
- 3 Surface between pedofossae with oval pits ..... *N. stammeri* Hirschmann & Z- Nicol, 1961
- Surface between pedofossae with reticulate pattern ..... *N. breviunguiculata* Willmann, 1949

## Genus *Trachycilliba* Berlese, 1903

**Type species.** *Uropoda splendida* Kramer, 1882.

### *Trachycilliba abantica* (Bal & Özkan, 2007)

*Uropoda abantica* Bal & Özkan, 2007: 43.

*Neodiscopoma abantica*.—Kontschán, 2013: 118.

*Trachycilliba abantica*.—**new combination.**

**Studied materials.** One female and one male specimens from soil and litter in the Ecological Garden of Nowshahr (51°57'50"N; 40°55'74"E), Mazandaran Province, northern Iran, altitude 30 m a.s.l., 10 June 2014, deposited in ACISTE.

**Note.** Bal and Özkan (2007) described this species as a member of *Uropoda* Latreille, 1808 *sensu lato* from Turkey. Kontschán (2013) reported it from Greece, and he transferred it to the genus *Neodiscopoma* Vitzthum, 1942, based on the species morphological characters, such as a dorsal shield with a strongly sclerotized median region elevated from other parts; and posteromarginal setae inserted on separate, individual platelets. The genera *Neodiscopoma* and *Trachycilliba* have a same type species, *Uropoda splendida* Kramer, 1882, therefore we placed the species within *Trachycilliba*. Herein, we report *T. abantica* from the Ecological Garden of Nowshahr in northern Iran near the Caspian Sea. This represents the third report of this species in the world.

## Acknowledgments

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# Description of a new troglomorphic species of *Charinus* Simon, 1892 from Brazil (Arachnida, Amblypygi, Charinidae)

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## Abstract

*Charinus taboa* **sp. n.** comprises the twenty-second species of the genus described for Brazil. The new species belongs to the eastern Brazilian group, in which all species have sucker-like gonopods. *Charinus taboa* **sp. n.** has a marked sexual dimorphism in the pedipalps as do other members of the genus in the country. The description of *Charinus taboa* **sp. n.** offers an opportunity to discuss some aspects of ecology, troglomorphism and conservation within the genus. A key to the eastern Brazilian species of *Charinus* is provided.

## Keywords

Neotropics, subterranean species, taxonomy, whip spider

## Introduction

Knowledge of the Amblypygi fauna remained low and fairly constant for many years until last decade of the 20<sup>th</sup> century, when studies and descriptions of whip spiders considerably increased in number (Harvey 2007). Yet, until 2015, the number of known species of *Charinus* in Brazil was just 11, but it has rapidly almost doubled over the

last year, reaching 21 described species (Vasconcelos and Ferreira 2016, Giupponi and Miranda 2016). The most specious and widely distributed group of Amblypygi is the genus *Charinus*. It is found throughout the tropics, including diverse types of habitat and even oceanic islands (Weygoldt 2000, Miranda and Giupponi 2011, Jocque and Giupponi 2012, Harvey 2013, Vasconcelos et al. 2013, 2014, Réveillion and Maquart 2015). However, the considerable richness of the genus (more than 60 species) allied with its wide distribution and distinct morphologies of the female genitalia suggest that the genus is not monophyletic (Weygoldt 1996, 2000, Harvey 2013).

Species of the genus *Charinus* are dependent on moist environments, and as other whip spiders, they are of nocturnal habit (Weygoldt 2000, Vasconcelos et al. 2013). Together with their flattened bodies, one of the characteristics of Amblypygi, *Charinus* species also have small body sizes (up to 16mm), which allow them to shelter in a range of microhabitats, such as trunks, bromeliads, rocks, subterranean cavities and termite nests (Weygoldt 2000, Jocque and Giupponi 2012, Vasconcelos et al. 2014, Réveillion and Maquart 2015). Synanthropic habitats can also house some species of *Charinus*, such as *C. vulgaris* Miranda and Giupponi, 2011, and the parthenogenetic species, *Charinus acosta* (Quintero, 1983) and *Charinus ioanniticus* (Kristcher, 1959) (Armas 2005, Weygoldt 2005).

In Brazil, *Charinus* species typically use caves as habitat. Within the 21 known species of the genus in the country, 13 were described from individuals collected in caves: *Charinus acaraje* Pinto-da-Rocha, Machado and Weygoldt, 2002, *Charinus mysticus* Giupponi and Kury, 2002, *Charinus troglobius* Baptista and Giupponi, 2002, *Charinus eleonora* Baptista and Giupponi, 2003, *Charinus potiguar* Vasconcelos, Giupponi and Ferreira, 2013, *Charinus jibaossu* Vasconcelos, Giupponi and Ferreira, 2014, *Charinus caatingae* Vasconcelos and Ferreira, 2016, *Charinus iuiu* Vasconcelos and Ferreira, 2016, *Charinus ricardoi* Giupponi and Miranda 2016, *Charinus carajas* Giupponi and Miranda 2016, *Charinus orientalis* Giupponi and Miranda 2016, *Charinus ferreus* Giupponi and Miranda 2016 and *Charinus bichuetteae* Giupponi and Miranda 2016. Among these species, *C. troglobius*, *C. eleonora*, *C. caatingae* and *C. ferreus* represent hitherto the only strictly cavernicolous species in the country. Herein, a new species of *Charinus* is described from limestone caves located in Sete Lagoas, state of Minas Gerais, Brazil. Moreover, the possible troglotic status of this species is discussed.

## Methods

The specimens were collected through visual searches throughout floors and walls of the caves. All specimens were captured with a fine forceps and placed in vials containing 70% ethanol.

The description of the species was based on the entire type material. Measurements and general terminology were based on the proposals of Quintero (1981). The terminology of pedipalps and legs followed Harvey and West (1998). The tarsus as defined by Harvey and West (1998) is divided here into tarsus and claw (apotele), as there is no fusion of these two segments in Charinidae. Measurements of the pedipalps were



taken between the condyles of each segment. Measurements were taken of the entire type-series (quantity indicated as “n”), presenting first their mean values followed by the range of variation in parentheses. The terminology of the structures of male gonopods followed Giupponi and Kury (2013).

The following abbreviations are used:

<b>BT</b>	basitibia;
<b>DT</b>	distitibia;
<b>GO</b>	genital operculum;
<b>Fi</b>	fistula (gonopod tube);
<b>Pi</b>	processus internus;
<b>LaM</b>	lamina medialis;
<b>LoD</b>	lobus dorsalis;
<b>LoL1</b>	lobus lateralis primus;
<b>LoL2</b>	lobus lateralis secundus.

Photographs were taken using a Leica M205A stereomicroscope with the software Leica Application Suite Automontage. Illustrations of the male and female gonopods were made using a camera lucida coupled to a Leica MDLS phase contrast microscope.

The specimens were deposited in the following institution collections:

<b>MNRJ</b>	Museu Nacional, Rio de Janeiro, Brazil;
<b>ISLA</b>	Seção de Invertebrados Subterrâneos, Coleção de Zoologia of the Universidade Federal de Lavras, Minas Gerais, Brazil;
<b>CAVAISC</b>	Fundação Oswaldo Cruz, Instituto Oswaldo Cruz, Rio de Janeiro, Brazil
<b>DNS</b>	Geographical coordinates are given in Degrees, Minutes and Seconds.

## Taxonomy

### Charinidae Quintero, 1986

#### *Charinus* Simon, 1892

**Type-species.** *Phrynus australianus* L. Koch, 1867, by original designation.

#### *Charinus taboa* sp. n.

<http://zoobank.org/5F363344-B51E-4F55-872E-09AB75E8F2F3>

Figs 1–23

**Type-locality.** BRAZIL, Minas Gerais: Sete Lagoas, 19°28'29.68"S, 44°19'41.31"W, Taboa Cave and BR 24 cave (19°27'59.89"S 44°19'48.47"W)

**Type-material.** Holotype male (MNRJ 09091) from Taboa cave (19°28'29.68"S, 44°19'41.31"W), Sete Lagoas, Minas Gerais, Brazil, 15 Sept. 2005, R. L. Ferreira leg. Paratypes: juvenile female (MNRJ 09092), juvenile female (MNRJ 09092), female (ISLA 4019), female (ISLA 4020), female (ISLA 4021), male (ISLA 4022), male (ISLA 4023), juvenile male (ISLA 4024), juvenile male (ISLA 4030) with the same data as holotype, female and male (CAVAISC-ARAC 0007) from BR 24 cave (19°27'59.89"S, 44°19'48.47"W), Sete Lagoas, Minas Gerais Brazil, 22 Jun. 2015, F. Bondezan leg. and female (CAVAISC-ARAC 0008) from BR 24 cave, Sete Lagoas, Minas Gerais Brazil, 18 Dec. 2015, F. Bondezan leg.

**Diagnosis.** *Charinus taboa* differs from other species of the genus by the following combination of characteristics: frontal process with thickened apex; median eyes reduced, with flattened tubercle; lateral eyes not developed and without pigmentation (little pigmentation in smaller individuals); tritosternum with a slightly forked apex; pedipalps sexually dimorphic; femur of the pedipalp with 4–5 dorsal spines (typically 5) and 5–6 ventral spines (typically 5); patella of the pedipalp with 6–7 dorsal spines (typically 6) and 4 ventral spines; distitibia of the leg IV with 16 trichobothria; female gonopod sucker-like, with irregular opening and edges with a small fold; male gonopod with pairs of Pi and LoL1 emerging from each side of the Fi with thin prolongations, and pairs of LoD and LoL2 claw-shaped emerging from the interior of the upper portion of Fi.

**Description.** *Carapace* (Figs 1–3, 9): Flattened. Wider than long (ratio length/width a little less than 3/4). Anterior margin rounded with corners flattened downwards. Six strong setae on the anterior margin projected upwards (one extra seta is found centrally in a female), the central two setae usually located directly in front of the tubercle of the median eyes. Frontal process triangular in shape, with thickened apex and visible in dorsal view. Carina begins at the corners of the anterior margin and extends from the coxae of leg II to the corners of the posterior margin. Median eyes reduced, with flat tubercle. Lateral eyes not very developed, without pigmentation (less pigmentation in smaller specimens) and with one seta posterior to each triad. Frontal hump present at each side, starting just at the front of the lateral eyes and ending in a depression located at each side of the carapace. Fovea located posterior to the center, from which radiate two pairs of furrows in anterior and posterior orientation like an "X". Median depression located on each side between these two pairs of furrows. A thin furrow follows medially from the median eye tubercle and reaches the posterior margin. Punctuations arranged in lines and spots, more densely in the anterior region.

*Sternum* (Figs 8, 11): Tri-segmented with all segments sclerotized and convex. Tritosternum projected anteriorly, elongated, cone-shaped, with slightly forked apex, with one apical pair of strong setae and three median strong setae in the holotype, and one apical, one medial and one basal pair of strong setae in the paratypes. Few setae along the tritoesternum. Second segment (mesosternum) rounded, with one strong seta at each upper corner and few setulae encircling the base. Third segment (metasternum) rounded, with one strong seta at each upper corner and few setulae encircling the base. The segments are separated from each other approximately by the diameter of the mesosternum.

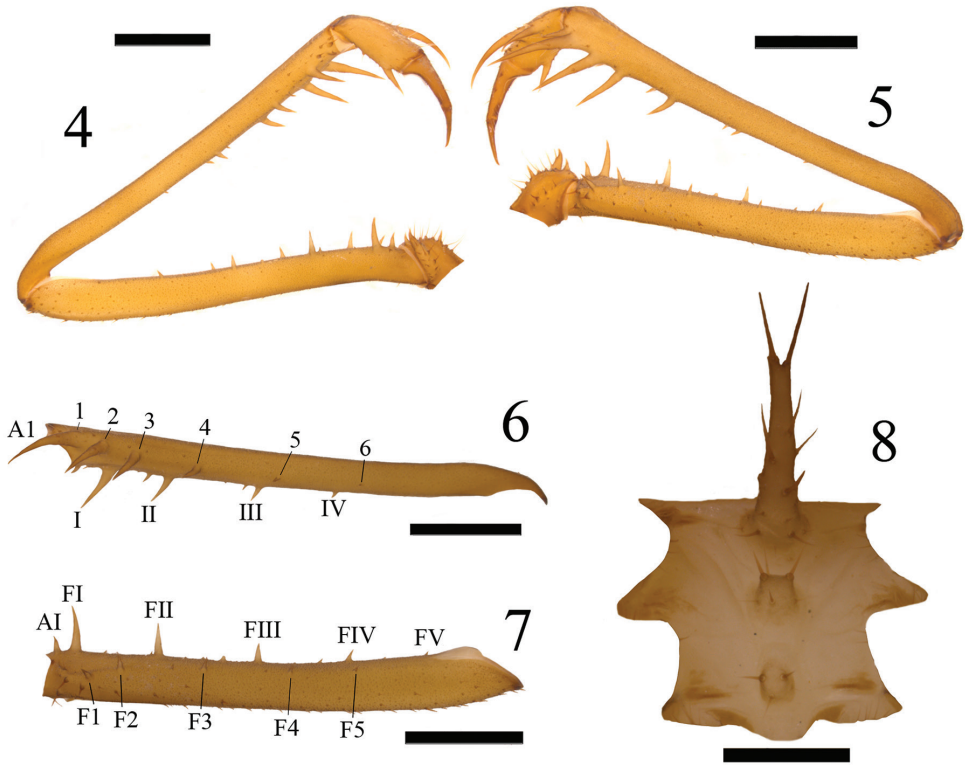


**Figures 1–3.** *Charinus taboa* sp. n. Holotype: **1** Habitus. **2** Carapace **3** Frontal view of the carapace and frontal process. Scale bars: **1** = 2 mm; **2, 3** = 1 mm.

*Abdomen* (Fig. 1): Oblong, with punctuations barely distinguishable.

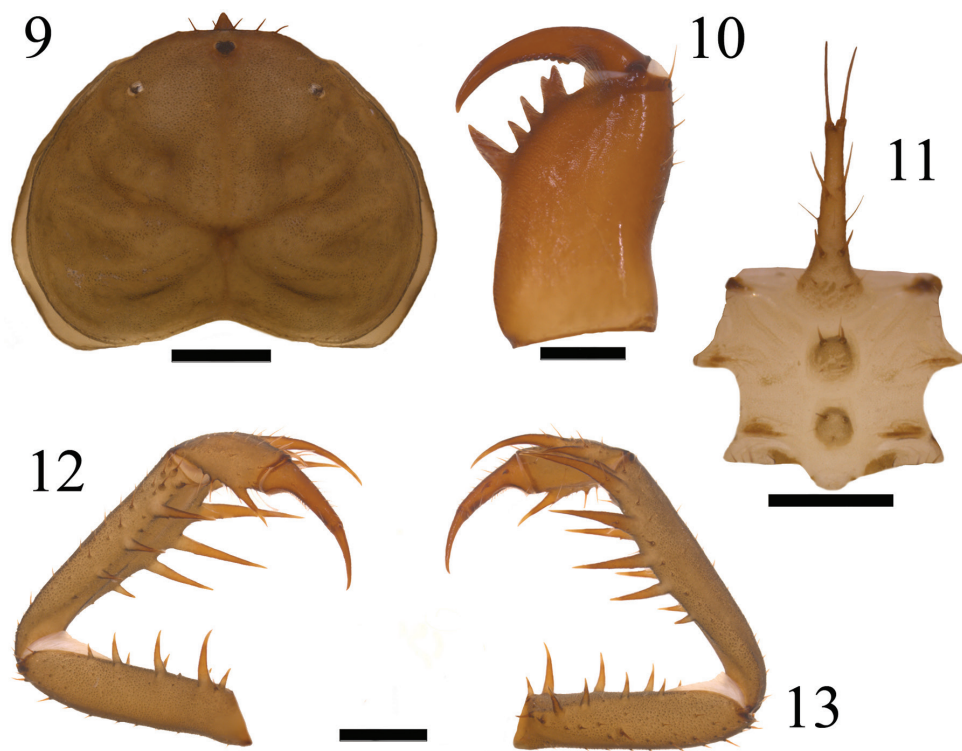
*Chelicera* (Fig. 10): Cheliceral furrow with 4 inner teeth. The distal tooth is bifid, the distal cusp being larger than the proximal. Teeth length:  $IV > Ia > Ib = II > III$ . Claw with 8-9 denticles. Dorsal setae located distally and in the inner side of the chelicerae.

*Pedipalp* (Figs 4–7, 12, 13): Trochanter: ventral spiniform apophysis pointed forwards with a series of subequal setiferous tubercles; two spines of subequal length located aligned on the prolateral face, the first being near the medial region and the



**Figures 4–8.** *Charinus taboa* sp. n. Holotype: **4** Right pedipalp in ventral view **5** Right pedipalp in dorsal view **6** Patella of the pedipalp in dorsal view with spines indicated **7** Femur of the pedipalp in dorsal view with spines indicated **8** Sternum. Scale bars: **4–7** = 2 mm; **8** = 500  $\mu$ m.

second dorsally to the projection of the apophysis and close to the femur; three setae aligned between the spines and two located basally to the first spine; dorsal oblique series of strong setae. Femur: dorsal portion with three strong setiferous tubercles on the basal region, one being located more ventrally; several strong setae along the segment; four-five dorsal spines (typically five) decreasing in size:  $F1 > F2 > F3 > F4 > F5$ ; five-six ventral spines (typically five) of sizes:  $FI > FII > FIII > A1 > FIV > FV$ ; some secondary spines are present in males between the spines on the dorsal and ventral sides. Patella: some strong dorsal setae between the spines; six-seven dorsal spines (typically six) of sizes:  $1 > 2 > 3 > 4 > A1 > 5 > 6$ ; large ventral setiferous tubercles located distally: four ventral spines of sizes:  $I > II > III > IV$ ; some secondary spines between the ventral spines in males. Tibia: strong dorsal setae; two dorsal spines, the second being approximately two times larger than the first; strong ventral setae on the basal portion; one ventral spine located on the distal half of size slightly smaller than the dorsal spine one. Tarsus: strong dorsal setae and some long ventral setae; two dorsal spines on the cleaning organ, the second being approximately two times larger than the first spine. Cleaning organ occupies about half the length of the article. Claw (apotele): long with sharp curved tip.



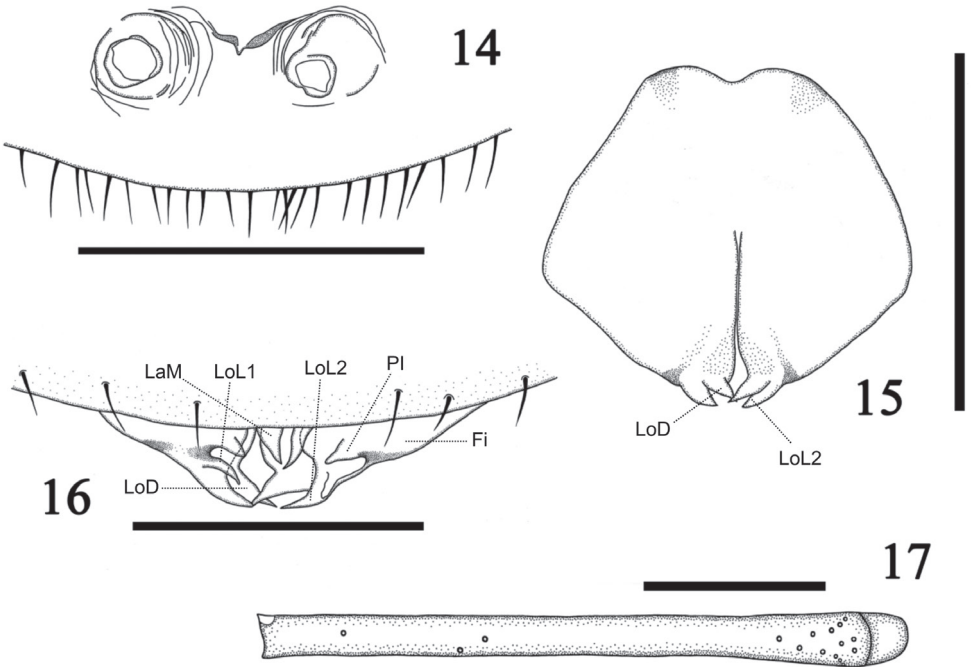
**Figures 9–13.** *Charinus taboa* sp. n. Female paratype: **9** Carapace **10** Right chelicerae **11** Sternum **12** Right pedipalp in ventral view **13** Right pedipalp in dorsal view. Scale bars: **9, 12, 13** = 1 mm; **10, 11** = 500  $\mu$ m.

*Legs*: all densely setose. Femur lengths:  $I > III > II > IV$ . Leg I: tibia with 23 articles and tarsus with 41 articles. Leg IV: basitibia with four pseudo-articles and one trichobothrium located basally on the last article. Distitibia (Fig. 17) with three basal and 13 distal trichobothria; frontal and caudal series with five trichobothria each. Basitibia-distitibia length:  $BT1 > DT > BT4 > BT3 > BT2$ . Ratio tarsus/metatarsus approximately  $3/4$ . Tarsus tetramerous.

*Color* in live specimens (Figs 18, 19): body light brown. In alcohol (Fig. 1): body yellowish; some specimens exhibit slightly reddish coloration on the carapace, pedipalps, chelicerae and legs.

*Male genitalia* (Figs 15, 16): GO rounded with few scattered setae. Genitalia a little wider than long. Longitudinal split occupies about half of the genital organ. Fi exceeds the genital operculum margin. Sclerotized band surrounds each side of the Fi, reaching the Pi and LoL1. Pair of Pi and LoL1 emerges in thin prolongations from each side of the Fi. Pair of LoD and LoL2 claw-shaped emerges from the interior of the upper portion of Fi, with LoL2 being ventral to the LoD. Pair of LaM claw-shaped and smaller, located ventrally to the Fi.





**Figures 14–17.** *Charinus taboa* sp. n. Female paratype: **14** Gonopod. Holotype: **15** Dorsal view of the gonopod **16** Ventral view of the gonopod with structures indicated: Fi = fistula (gonopod tube), Pi = processus internus, LaM = lamina medialis, LoD = lobus dorsalis, LoL1 = lobus lateralis primus, LoL2 = lobus lateralis secundus **17** Distitibia of the right leg IV. Scale bars: **14, 16** = 500 µm; **15, 17** = 1 mm.

**Table 1.** Measurements (mm) of body parts of the specimens of *Charinus taboa* sp. n.

		Males (n = 5)	Females (n = 5)
Total length		8.74 (6.56–11.12)	9.14 (6.02–10.85)
Cephalotorax	Length	3.31 (2.64–4.35)	3.21 (2.55–3.51)
	Width	4.69 (3.80–5.82)	4.63 (3.33–5.24)
Pedipalp	Femur	4.56 (2.23–9.26)	3.11 (2.01–3.60)
	Patella	5.02 (2.62–10.19)	3.30 (2.14–3.92)
	Tibia	1.43 (0.98–2.06)	1.34 (0.80–1.56)
	Tarsus	1.03 (0.78–1.42)	0.96 (0.66–1.08)
	Claw	0.72 (0.68–0.94)	0.74 (0.44–0.86)

*Female genitalia* (Fig. 14): Rounded genital operculum margin with many strong setae. Gonopods sucker-like, barrel shaped and slightly wider than long. Irregular gonopods opening, with edges with a small fold retracted in a portion between the gonopods and the operculum margin, and with a bottleneck below the edges. Gonopods separated from each other by a distance smaller than the diameter of each one and from the margin of the operculum by a distance larger than its length.





**Figures 18–19.** *Charinus taboa* sp. n. **18** Male inside the Taboa cave **19** Female preying a moth (Noctuidae).

**Etymology.** The specific epithet is treated as a noun in apposition and refers to the name of the cave (Taboa) where most of the specimens were collected.

**Distribution.** The new species is known from the Taboa and BR 24 caves, state of Minas Gerais, Brazil.

**Ecology.** Amblypygids perform their vital activities, such as mating and feeding, in nocturnal periods. The most important sensory organ used by whip spiders for capturing prey is the antenniform leg, while the eyes are most important for avoiding light (Weygoldt 2000, Pinto-da-Rocha et al. 2002). This way, amblypygids can be considered pre-adapted to subterranean life, since they are able of searching for food in a completely darkness.

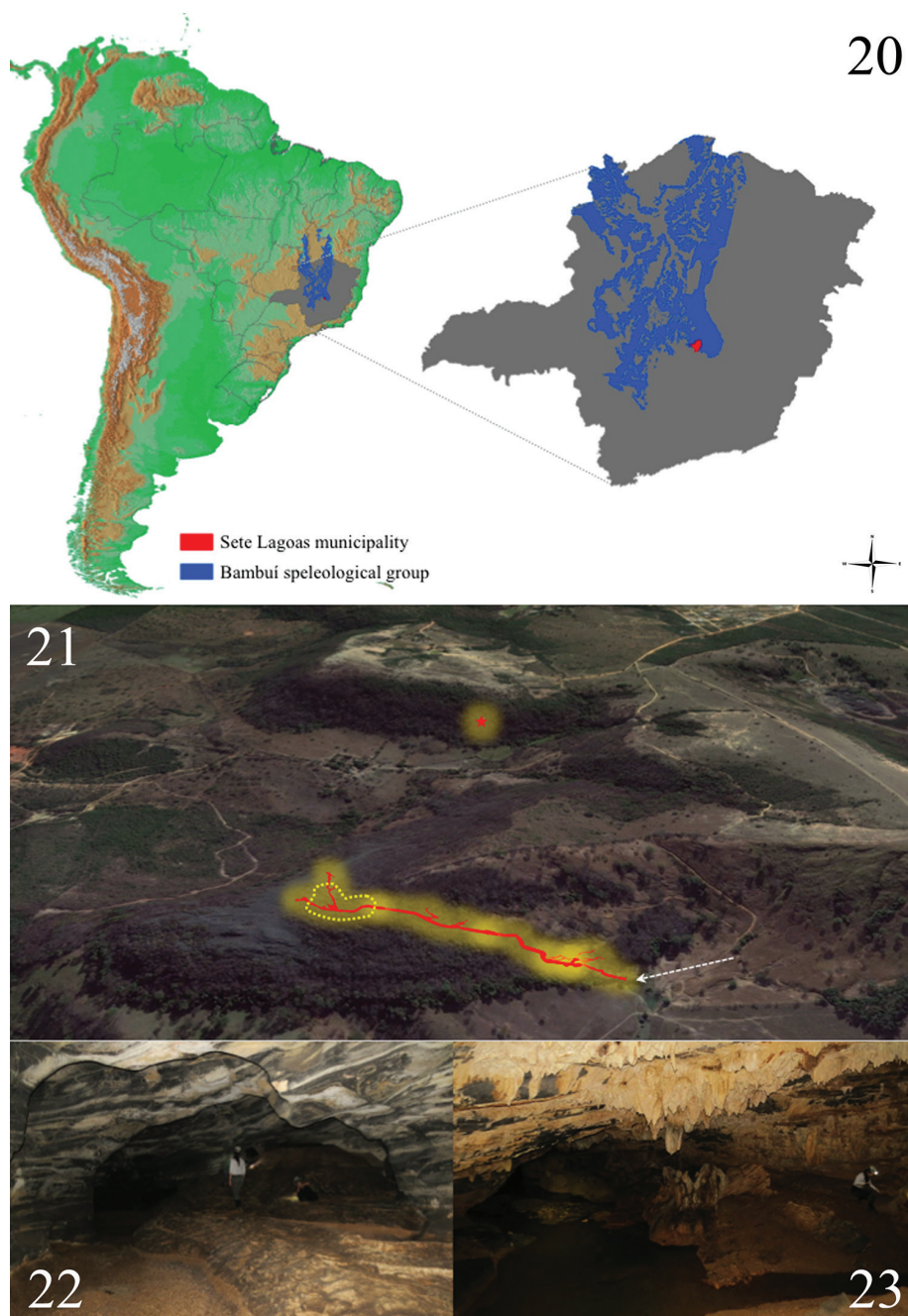
Eyes in Amblypygi are also important for adjusting to circadian rhythms (Weygoldt 2000). After many generations living in a subterranean habitat, some hypogean animals might have their activity period modified, as shown for some species of fish (Menna-Barreto and Trajano, 2015). This possible change in behavior may have occurred in *Charinus taboa*, as one specimen was observed preying on a Noctuidae (Lepidoptera) during the day (Fig. 19). Nevertheless, we cannot discard the possible scenario where the common ancestral of the clade where *C. taboa* belongs was a species that have diurnal activity of alimentation.

Contrarily to that observed, moths were considered by Weygoldt (2000) as typical preys of *Heterophrynus* Pocock, 1894, which are agile “sit and wait” predators, as opposed to animals of small body size, as *Charinus*, which behave as active predators. In ground habitats, amblypygids also act as one of the largest predators in these environments, as the fauna of underground cavities consists mostly of small arthropods (Culver and Pipan 2009). Other invertebrates, as crickets and cockroaches, have been cited as potential preys of *Charinus* in caves (Vasconcelos et al. 2013, Vasconcelos and Ferreira 2016).

Specimens of *C. taboa* were only found in two caves (Taboa cave and BR 24 cave), both located in the Bambui speleological group and near the city of Sete Lagoas (Fig. 20), in a zone with pronounced anthropization (Fig. 21). The external native vegetation was quite modified, with fragments of vegetation associated only with rocky outcrops, which comprises inappropriate areas for agriculture. Nevertheless, many outcrops were altered anthropically or completely destroyed by mining activities. Tens of caves were target of biospeleological inventories in the region where Taboa and BR 24 caves are located (R. Ferreira, data not published). Nonetheless, individuals of *C. taboa* were not found in other localities than the cited caves. Such caves are quite close to each other, with the distance between them less than 500 meters (both caves are associated to a continuous limestone outcrop) (Fig. 20). It is important to point that although both caves (Taboa and BR-24) are not connected by macro-spaces, it is plausible to assume the existence of meso-caverns in between them. Such small spaces would certainly allow the movements of individuals through the underground between those caves.

The BR-24 cave is a small cave (33,8 meters long), with a single entrance and an isolated chamber in its deepest portion, where the specimens were found. This chamber is quite moist, even during the dry season. In total, 6 specimens were found in the





**Figure 20–23.** **20** Locality of Sete Lagoas (municipality where are located the Taboa and BR 24 caves) in the state of Minas Gerais, Brazil. The blue area corresponds to the Bambuí limestone group and the red area correspond to the Sete Lagoas municipality **21** Location of the Taboa cave (the arrow indicates the main entrance of Taboa cave and the circle the location where individuals of *C. taboa* were found) and BR-24 cave (star represents the entrance) **22** Portion of the Taboa cave where specimens were collected **23** Portion of the Taboa cave with a watercourse where most of the specimens were found.

dry season and only one specimen was observed in the rainy season. Specimens of *C. taboa* were observed in the cave walls and ceiling, always in the deepest portion of the cave. Potential preys include moths and crickets.

During the visit to the Taboa cave (which has around 800 meters long), about 15 adults and 10 juveniles were observed. The adults were mainly found between speleothems on walls and ceiling of the cave, while juveniles were seen frequently under rocks. This behavior of sheltering among speleothems and under rocks may eventually mean a response to pressure of cannibalism or predation, since other predators of bigger size (as spiders of the genus *Isoctenus* Bertkau, 1880) cohabit the cave. This type of behavior was also registered in *C. potiguar* and in juveniles of *Heterophrynus cheiracanthus* (Gervais, 1844) in the night (Ladle and Velander 2003). All individuals of *C. taboa* were found in the inner portion of the cave, near to a large watercourse (Figs 22, 23). Similar preference was also observed in *C. troglobius* and *C. eleonora* (Baptista and Giupponi 2002, 2003).

#### Key to eastern Brazilian species of *Charinus* (modified from Miranda and Giupponi 2011)

- 1 Median eyes absent ..... *C. troglobius*
- Median eyes present ..... 2
- 2 Second and third sternal sclerites flattened and twice as wide as long (Espírito Santo: Domingos Martins) ..... *C. montanus*
- Second and third sternal sclerites convex and roundish ..... 3
- 3 Distitibia of leg IV with 18 trichobothria ..... 4
- Distitibia of leg IV with 16 trichobothria ..... 11
- 4 Patella of the pedipalp with 2 ventral spines ..... 5
- Patella of the pedipalp with 3 or more ventral spines ..... 6
- 5 Lateral eyes triads with pigmentation (Bahia: Santa Luzia, Gruta Pedra do Sino Cave) ..... *C. acaraje*
- Lateral eyes triads without pigmentation (Rio Grande do Norte: Felipe Guerra, Buraco Redondo Cave) ..... *C. potiguar*
- 6 Median eyes tubercle indistinct (Minas Gerais: Itacarambi, Olhos d'Água Cave) ..... *C. eleonora*
- Median eyes tubercle distinct ..... 7
- 7 Lateral eyes underdeveloped (Bahia: Várzea Nova: Fazenda Jurema Cave) ..... *C. caatingae*
- Lateral eyes developed ..... 8
- 8 Patella of the pedipalp with 3 ventral spines ..... 9
- Patella of the pedipalp with 4 or 5 ventral spines ..... 10
- 9 Femur of the pedipalp with 3 or 4 dorsal spines (Bahia: Iuiu: Lapa do Baixão Cave) ..... *C. iuiu*
- Femur of the pedipalp with 5 or 6 dorsal spines (Minas Gerais: Arcos: Gruta da Cazanga) ..... *C. jibaossa*

- 10 Tarsus of the pedipalp with 3 dorsal spines (Bahia: Gentio do Ouro, Encantados Cave) ..... ***C. mysticus***
- Tarsus of the pedipalp with 2 dorsal spines (São Paulo: Ilha Bela) ... ***C. asturius***
- 11 Lateral and median eyes developed with high tubercle (Espírito Santo: Serra) ..... ***C. brasiliensis***
- Lateral and median eyes underdeveloped with low tubercle (Minas Gerais: Sete Lagoas: Taboá Cave) ..... ***C. taboá* sp. n.**

## Discussion

As proposed by Weygoldt (2005, 2006, 2008), species of *Charinus* can be divided into four groups based on the morphology of the female gonopods, including species with “sucker-like” gonopods (*Charinus brasiliensis* group), “cushion-like” gonopods (*Charinus australianus* group), a group of species with “finger-like” gonopods (*Charinus bengalensis* group), and finally, the group represented by *Charinus seychellarum*, in which the gonopods were totally lost. In Brazil, the species distributed on the eastern side are included in the *C. brasiliensis* group, while species distributed in the Amazon region are considered part of the *C. australianus* group. *Charinus taboá* is placed in the first group in conjunction with *C. brasiliensis* Weygoldt, 1972, *C. montanus* Weygoldt, 1972, *C. asturius* Pinto-da-Rocha, Machado & Weygoldt, 2002, *C. acaraje*, *C. mysticus*, *C. troglobius*, *C. eleonora*, *C. potiguar*, *C. jibaossu*, *C. caatingae* and *C. iuiu*. *Charinus schirchii* (Mello-Leitão, 1931) is also located in eastern Brazil, but it is considered a *species inquirenda* as the holotype specimen has been lost and so its morphological characteristics and grouping cannot be confirmed (Pinto-da-Rocha et al. 2002). Species of this group also differ from the other western species by being larger, presenting chelicera with a higher number of teeth and basitibia of leg IV with four pseudoarticles (Giupponi and Miranda 2016).

Among the species from southeast Brazil, another common character is found, a sexual dimorphism in the pedipalps (Table 1, Figs 18, 19). With exception of the troglobite species *C. eleonora* (which is distributed further north), males of *C. taboá* and all other *Charinus* species that occur in this region of the country (*C. montanus*, *C. brasiliensis*, *C. asturius* and *C. jibaossu*) have longer pedipalps than the females (Pinto-da-Rocha et al. 2002, Vasconcelos et al. 2014, Weygoldt 1972). The presence of this characteristic among these species may indicate that they share the same recent common ancestor. Or, alternatively, organisms of these species might have undergone similar selective pressures in the past, which could have led to homoplasy in this character. Therefore, there is pressing need for a phylogenetic analysis of Brazilian *Charinus* species to understand these questions of relatedness between species.

*Charinus taboá* differs from *C. montanus*, *C. brasiliensis*, *C. asturius* and *C. jibaossu* by having less developed eyes, and with the exception of *C. brasiliensis*, five thricobotria instead of six in each series of the basitibia of leg IV (Fig. 17). *Charinus taboá* also differs from *C. montanus* in the shape of the segments of the sternum. While the

second and third segments of the sternum of *C. taboa* are rounded (Figs 8, 11), in *C. montanus* these segments are flattened. *Charinus taboa* differs from *C. jibaossu* by having four spines in the ventral side of the patella (Fig. 6) instead of three. The quantity of spines on the others segments of the pedipalps is similar among many of the cited species, which makes it difficult to separate them by this characteristic alone (Weygoldt 1972, Pinto-da-Rocha et al. 2002, Vasconcelos et al. 2014).

*Charinus taboa* differs from *C. acaraje*, *C. troglobius*, *C. eleonora*, *C. potiguar*, *C. caatingae* and *C. iuiu* mainly by the fact that these species have shorter pedipalps, with fewer amount of spines on the femur and patella, and from *C. mysticus* and *C. caatingae* by the presence of three spines on the tarsus of the pedipalps. *Charinus mysticus*, *C. acaraje*, *C. eleonora*, *C. potiguar*, *C. caatingae* and *C. iuiu* also have the frontal and caudal series of the leg IV with six thricobotria each, *C. eleonora* has a pointed frontal process, and in *C. troglobius*, the tritosternum is lacking the typical cone shape (Baptista and Giupponi 2002, 2003, Giupponi and Kury 2002, Pinto-da-Rocha et al. 2002, Vasconcelos et al. 2013, Vasconcelos and Ferreira 2016).

The morphologies of the male gonopod are quite variable among *Charinus* species; however, in dorsal view the shapes of the genital organ and LoD of *C. taboa* (Fig. 15) are similar to those in *C. eleonora*. The female gonopod of *C. taboa* (Fig. 14) has the width larger than its length, which makes it similar to those of *C. asturius*, *C. eleonora* e *C. mysticus*. Besides that, *C. taboa* presents its gonopod with the edges similar to that of *C. mysticus*, yet its shape is more irregular (Pinto-da-Rocha et al. 2002, Giupponi and Kury 2002, Baptista and Giupponi 2003).

The newly described species presents poorly developed eyes, lighter coloration than other non-troglobite species of *Charinus*, and is, to our knowledge, restricted to only two caves, which make plausible its status of troglobitic. Different degrees of troglomorphisms may appear due to changes in environmental conditions and not necessarily depends on cave occupancy by the organism. According to Weygoldt and Van Damme (2004), if a given region goes through changes in climate and consequently in humidity, organisms adapted to the anterior conditions can retreat in caves, which may result in troglomorphisms after some time, such as reduction in eye size and coloration, and elongation of legs and other appendages. Changes engendered by the restricted life in the interior of subterranean habitats in *Charinus* are the reduction in eye size, being completely absent in some cases, lightening of the color of the cuticle, change in the position of the pedipalps, being vertical in relation to the body, and elongation of the spines on the pedipalps (Baptista and Giupponi 2002, Baptista and Giupponi 2003, Weygoldt and Van Damme 2004, Delle Cave et al. 2009).

There are few troglobitic species of *Charinus* around the world: four in Brazil (*C. troglobius*, *C. eleonora*, *C. caatingae*, *C. ferreus*), two in Venezuela (*Charinus troncho-nii* (Ravelo, 1975) and *Charinus bordoni* (Ravelo, 1977)), and three in the Arabian Peninsula (*Charinus socotranus* Weygoldt, Pohl and Polak, 2002, *Charinus stygochthobius* Weygoldt and Van Damme, 2004, *Charinus omanensis* Delle Cave, Gardner and Weygoldt, 2009). *Charinus stygochthobius* represents the most troglomorphic species of those already described, since it lacks all its eyes, its cuticle is almost transparent, it has



the pedipalps forming an angle of  $45^\circ$  in relation to the body and long spines on the pedipalps (Weygoldt and Van Damme 2004). *Charinus troglobius* presents total absence of the median eyes and small eyespots replacing the lateral eyes, depigmentation of the body, rotation of the pedipalps, as occurs to *C. stygochthobius*, and elongated spines (Baptista and Giupponi 2002). *Charinus eleonorae* has reduced lateral eyes, eyespots in the place of median eyes, light coloration, rotation of the pedipalps and elongated spines (Baptista and Giupponi 2003). *Charinus caatingae* presents lateral eyes reduced to eyespots, light pigmentation, and rotation of pedipalps in some individuals, being the least troglomorphic species in the country. *Charinus ferreus* presents almost complete loss of eyes and little coloration of the cuticle.

In the case of *C. taboa*, the eyes are still present, but they are smaller than those of most *Charinus* species in Brazil. In addition, this species has lighter coloration of the body compared to other species. However, some specimens of *C. taboa* present pigmented lateral eyes with a lesser degree of reduction (Figs 2, 9). The varying degree of troglomorphy within a population is common, although not well studied, and may be a characteristic of various groups. One example is the isopod *Asellus aquaticus*, which has polymorphisms in the degree of pigmentation of the eyes and body, in the size of the sensory appendages and body proportions (Prevorcnik et al. 2004).

With a cladistic analysis unavailable, it is not possible to ascertain whether a species of *Charinus* is troglotic based solely on troglomorphic characters, since the species may have not been in a cave for sufficient time to develop morphological adaptations (beyond other factors, as the original size of the isolated population, species variability, etc.). Therefore, the condition of a given species of maintaining a viable population strictly inside caves should also be taken into account when deciding on the classification of a cave-dwelling species (Bolfarini and Bichuette 2015). This might be the case of *C. taboa*, which presents few troglomorphic characteristics, but is found within two caves located in a heavily deforested and human-modified landscape; the species seems to be unable to establish populations outside of its existing range. Yet, despite *C. taboa* has morphological characteristics suggesting the exclusive subterranean habit, more inventories are needed to confirm the non-occurrence of the species outside of these habitats.

Many species of *Charinus* in Brazil are highly vulnerable to extinction as a result of vast destruction of their habitat by deforestation or mining. *C. taboa*, which was recorded in only two very close caves, is considered rare and endemic. Thus, according to the laws of Brazil, this species may increase the biological importance of both the Taboa and BR-24 caves, and therefore ensures the continued preservation of those unique habitats.

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# Three new spider species of the genus *Pholcus* from the Taihang Mountains of China (Araneae, Pholcidae)

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## Abstract

In this study, three new species belonging to the genus *Pholcus*, collected from a forest of the Taihang Mountains, P. R. China, are described under the names of *Pholcus papillatus* **sp. n.** (male, female), *P. curvus* **sp. n.** (male, female) and *P. auricularis* **sp. n.** (male, female).

## Keywords

Hebei Province, Phlocinae, *Pholcus phungiformes*, species group, taxonomy

## Introduction

The spider family Pholcidae C. L. Koch, 1850 is the ninth largest spider family and, to date, 1461 species, belonging to 79 genera, have been reported (World Spider Catalog 2016). It has a worldwide distribution, but the highest diversity is concentrated in the tropical and subtropical regions. Phlocine spiders occupy a wide range of habitats in a variety of ecosystems, e.g., in buildings, under rocks, in caves, in leaf litter, and under leaves (Huber 2005a). Huber (2000, 2001, 2003a, b, c, 2005b, c, 2009a, 2011b) reported a large number of new species and revised many genera in this family. Based on cladistic analyses of morphological and molecular data and on qualitative character assessment (Huber 2011a; Dimitrov et al. 2013), Pholcidae was divided into five

subfamilies: Arteminae Simon, 1893, Modisiminae Simon, 1893, Ninetinae Simon, 1890, Pholcinae C.L. Koch, 1850 and Smeringopinae Simon, 1893; Pholcinae is the most species-rich subfamily.

*Pholcus* Walckenaer, 1805 is the largest genus in Pholcinae, with 318 described species which are mainly distributed in the Old World. *Pholcus* can be diagnosed by the following combination of characters: male chelicerae usually with three pairs of apophyses; bulb usually with uncus and appendix; epigynum usually strongly sclerotized and with ‘knob’ (Huber 2011b). These *Pholcus* spiders frequently live in houses, rock-crevices, caves and leaf litter, and most spin loose and irregular webs in sheltered areas. Recently, the genus has been studied by several scholars: Huber (2001) made a few taxonomic remarks; Dimitrov and Ribera (2007) and Dimitrov et al. (2008) revised and cladistically analyzed the Macaronesian *Pholcus* species; Zhang and Zhu (2009) reviewed 55 Chinese *Pholcus* species; Huber (2011b) revised the genus *Pholcus* and presented 254 species in 29 species groups; Yao and Li (2012) described 35 new species and also provided new illustrations for 45 known species from China and two species from Laos; Yao and Li (2013) described two new species from Laos; Yao, Pham and Li (2015) described five new species from Vietnam.

The spider genus *Pholcus* Walckenaer, 1805 exhibits a highly diversity in China. One hundred fifteen *Pholcus* species attached to nine species groups (*P. halabala* species group, *P. ponticus* species group, *P. crypticolens* species group, *P. zham* species group, *P. bidentatus* species group, *P. nagasakiensis* species group, *P. yichengicus* species group, *P. taishan* species group, and *P. phungiformes* species group) were recorded from China (Zhang and Zhu 2009; Tong and Ji 2010; Tong and Li 2010; Huber 2011b; Peng and Zhang 2011a, b; Yao and Li 2012; Liu and Tong 2015; World Spider Catalog 2016).

The Taihang Mountains are located in the northeastern China, between 34°34'N to 40°43'N and 110°14'E to 114°33'E. Fuping county, a county of Hebei Province, is located in the Taihang Mountains area. To explore the diversity of the arthropod in Fuping county, one survey was carried out in 2014. While examining the spider specimens collected from leaf litter in this survey, three new species belonging to *Pholcus* were found and are reported in the present paper. Detailed diagnosis, descriptions, and illustrations of these new taxa are presented.

## Material and methods

All specimens were preserved in 75% ethanol and examined, drawn, and measured under a Nikon SMZ1500 stereomicroscope equipped with a drawing tube. Photographs were taken with a Leica M205A stereomicroscope equipped with a Leica DFC550 Camera and LAS software (Ver. 4.6). Male and female genitalia were examined and illustrated after dissection. Epigyna were removed and treated in 10% warm solution of potassium hydroxide (KOH) before illustration. Left pedipalpi of male spiders were illustrated, except as otherwise indicated. All measurements are given in millimeters. Eye sizes were measured as the maximum diameter of the lens in dorsal or frontal view. Leg measurements are given as total length (femur + patella + tibia + metatarsus +



tarsus). Leg segments were measured on their dorsal side. Terminology and taxonomic descriptions follow Huber (2000, 2009b).

The following abbreviations are used in the text:

<b>AER</b>	anterior eye row;
<b>ALE</b>	anterior lateral eye;
<b>AME</b>	anterior median eye;
<b>MOA</b>	median ocular area;
<b>PER</b>	posterior eye row;
<b>PLE</b>	posterior lateral eye;
<b>PME</b>	posterior median eye;
<b>b</b>	bulb;
<b>e</b>	embolus;
<b>pa</b>	pseudo-appendix;
<b>pp</b>	pore plate;
<b>pr</b>	procursus;
<b>u</b>	uncus.

All specimens used in this studied are deposited in the Museum of Hebei University, Baoding, P. R. China (MHBUE).

## Taxonomy

### Family Pholcidae C. L. Koch, 1850

### Genus *Pholcus* Walckenaer, 1805

**Type species.** *Pholcus phalangioides* (Fuesslin, 1775)

### *Pholcus phungiformes* species group

The *P. phungiformes* group is largely distributed in northeastern China and the Korean Peninsula. Most species of this group have the following characters: carapace with vivid pattern, abdomen cylindrical, male chelicerae with proximal apophyses frontally, male palpal tibia with prolatero-ventral modification, procursus with dorsal spines, appendix absent, sometimes with pseudo-appendix (apophysis arising from uncus rather than from bulb, near usual position of appendix), epigynum sclerotized, with knob-shaped apophysis (Huber 2011). The pseudo-appendix of *P. exilis* auct, date, *P. wuling* auct, date, and *P. chicheng* auct, date, may be bifid, but the character needs further study. The three new *Pholcus* species are assigned as members of this group in possessing most of the characters of the *P. phungiformes* group.

***Pholcus papillatus* sp. n.**

<http://zoobank.org/3557D5D5-09B8-4733-BE13-767A8078CB1C>

Figs 1–4

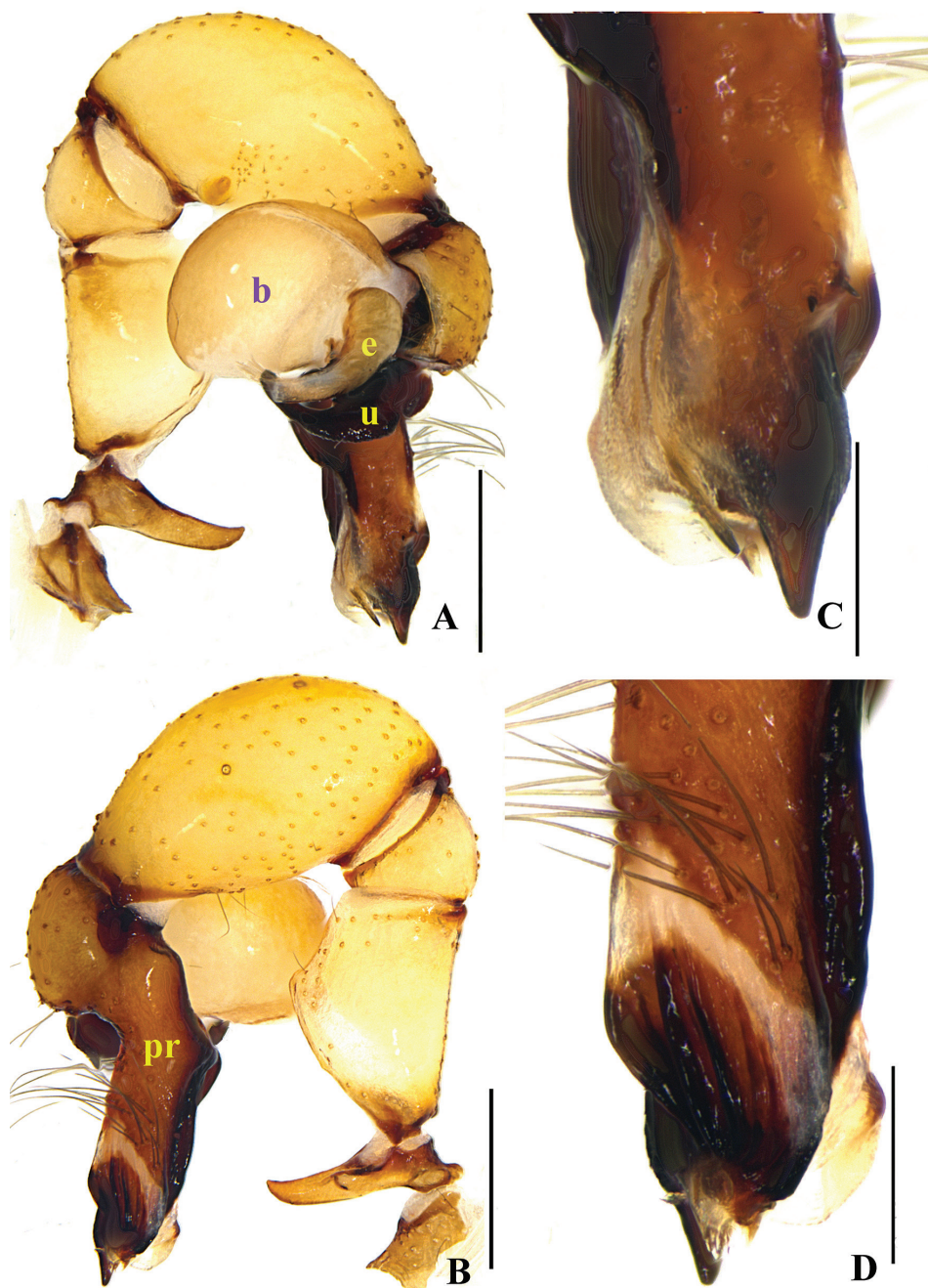
**Type material. Holotype:** male (MHBU), CHINA: Hebei Province, Fuping County, Longquanguan Town, Liaodaobei Village, 38°16'N, 114°17'E, alt. 1700 m, 6 August 2014, B.S. Zhang leg. **Paratypes:** 1 male and 3 females (MHBU), same data as in holotype.

**Etymology.** The specific name is from Latin word “*papillatus*”, in reference to the shape of epigynal apophysis; adjective.

**Diagnosis.** Narrow, long pseudo-appendix originating from the uncus (Fig. 2B). Distinguished from similar species with a pseudo-appendix by: palpal bulb with longer uncus, procurus with one spine-shaped projection and one hook-shaped membranous projection on tip (Figs 1A–D, 2A–B, 3A–D). The females of the new species are distinguished from females of similar species by the larger teat-shaped epigynal apophysis (Figs 2E, 4F).

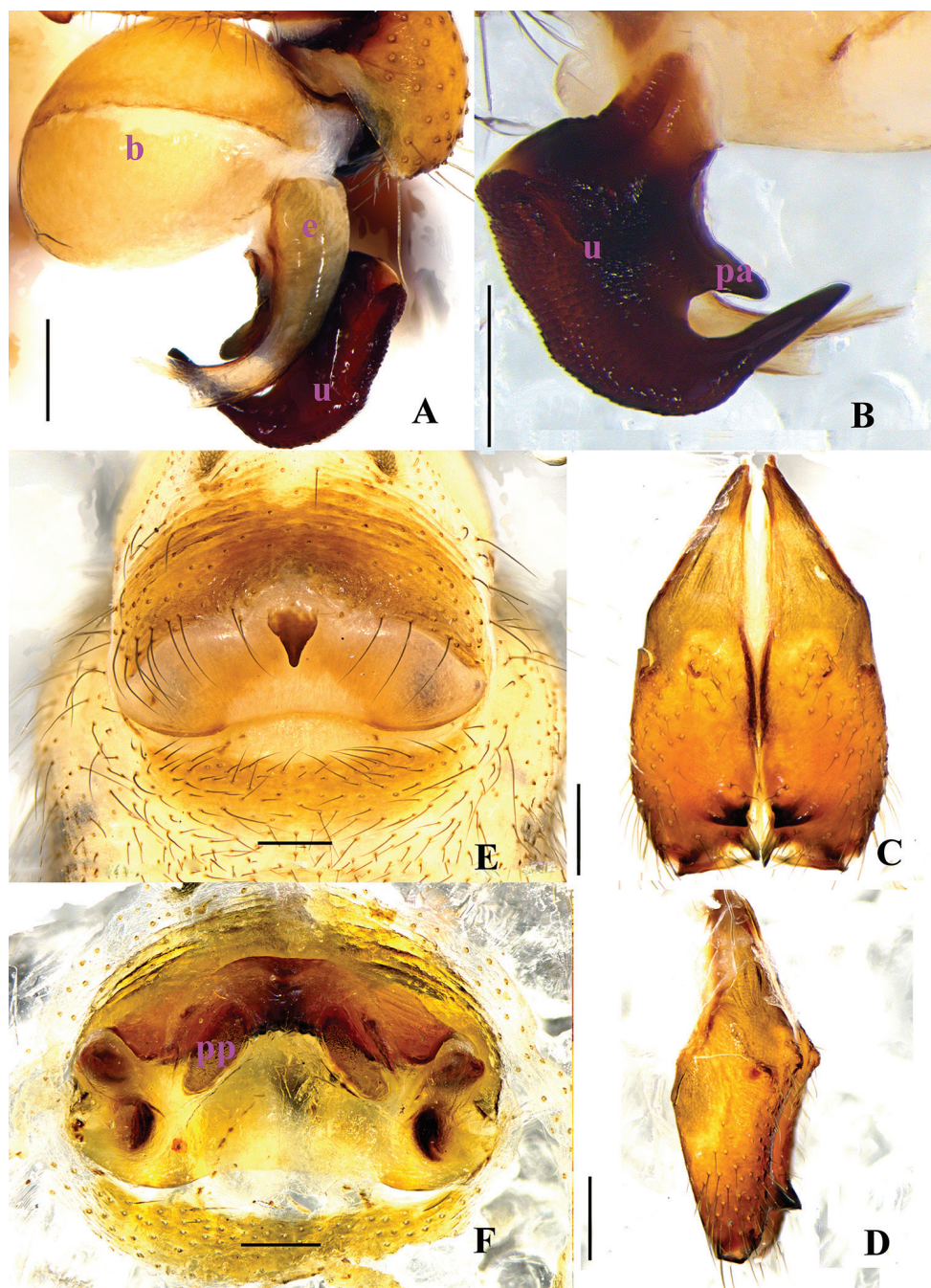
**Description. Male** (holotype): Total length 4.90 (5.10 with clypeus), prosoma 1.40 long, 1.52 wide, opisthosoma 3.36 long, 1.68 wide. Habitus as in Fig. 4A–C. Dorsal shield of prosoma pale grey, with dark brown radiated stripes and bands marginally; thoracic groove distinct; ocular area elevated, with short eye-stalks; ocular area yellow-brown, with a median dark brown band and two lateral dark brown bands beside PLEs, dorsal prosoma and ocular area both with dispersed dark brown spots, of them, two distinct spots behind PME; clypeus 0.23 high, dark brown, with light margin. Diameter AME 0.08, ALE 0.13, PME 0.12, PLE 0.13. Distance AME-AME 0.07, AME-ALE 0.10, PME-PME 0.24, PME-PLE 0.04, ALE-ALE 0.62, PLE-PLE 0.78. MOA 0.23 long, front width 0.23, back width 0.46. Chelicerae as in Figs 2C–D and 4D–E, with pair of black apophyses distally, pair of unsclerotized, small and nearly nipple-shaped apophyses proximolaterally and frontally. Labium and endites dark brown, distal part pale, labium wider than long (0.32/0.15). Sternum wider than long (1.04/0.77), dark brown, median part of sternum light. Legs long, brown, but dark brown on proximal parts of tibiae, and on distal parts of femora and tibiae, with whitish on subdistal parts of femora and tibiae. Measurements of legs: I 36.78 (9.59 + 0.59 + 9.69 + 15.43 + 1.48), II 26.81 (7.37 + 0.55 + 5.89 + 11.61 + 1.39), III 22.46 (6.55 + 0.61 + 5.42 + 8.59 + 1.29), IV 24.58 (7.06 + 0.53 + 5.74 + 9.89 + 1.36); tibia I L/d: 62. Leg formula: 1243. Opisthosoma pale grey, with dark spots dorsally and laterally. Pedipalpi illustrated in Figs 1A–D and 3A–D; trochanter with a long ventral apophysis; tibia with a small projection prolaterally; procurus simple proximally and complex distally, dorsal spines present; uncus with a pseudo-appendix, the tip of uncus long and bent; embolus weakly sclerotized.

**Female.** Generally similar to male. One specimen measured: total length 4.58 (4.80 with clypeus), prosoma 1.34 long, 1.46 wide, opisthosoma 3.09 long, 1.44 wide. clypeus 0.23 high. Diameter AME 0.06, ALE 0.10, PME 0.09, PLE 0.11. Distance AME-AME 0.08, AME-ALE 0.07, PME-PME 0.19, PME-PLE 0.04, ALE-ALE 0.50,

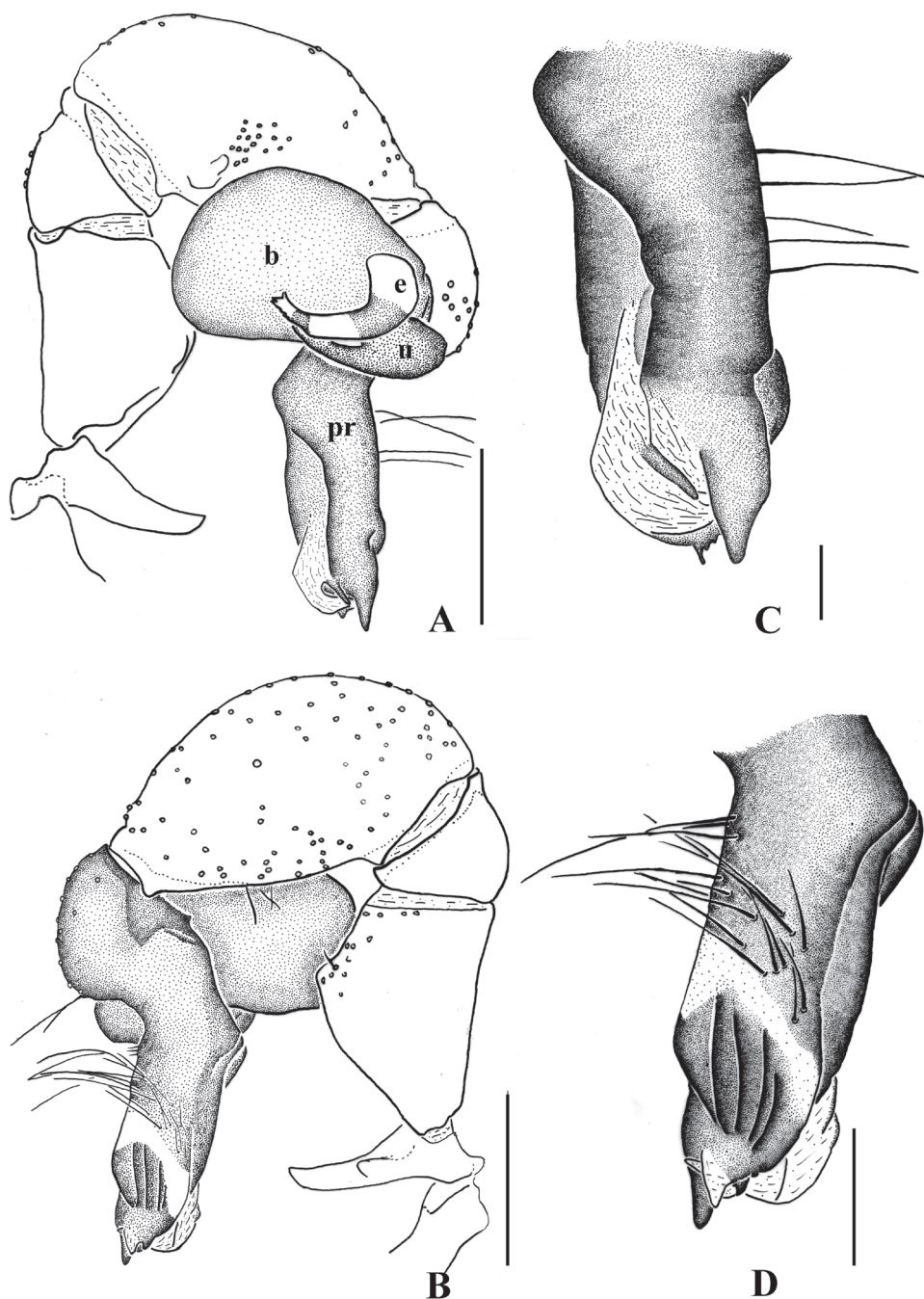


**Figure 1.** *Pholcus papillatus* sp. n., male holotype. **A–B** Pedipalpus (**A** prolateral view **B** retrolateral view) **C–D** Distal part of procurus (**C** prolateral view **D** retrolateral view). Scale bars: 0.2 mm (**C, D**); 0.5 mm (**A, B**).



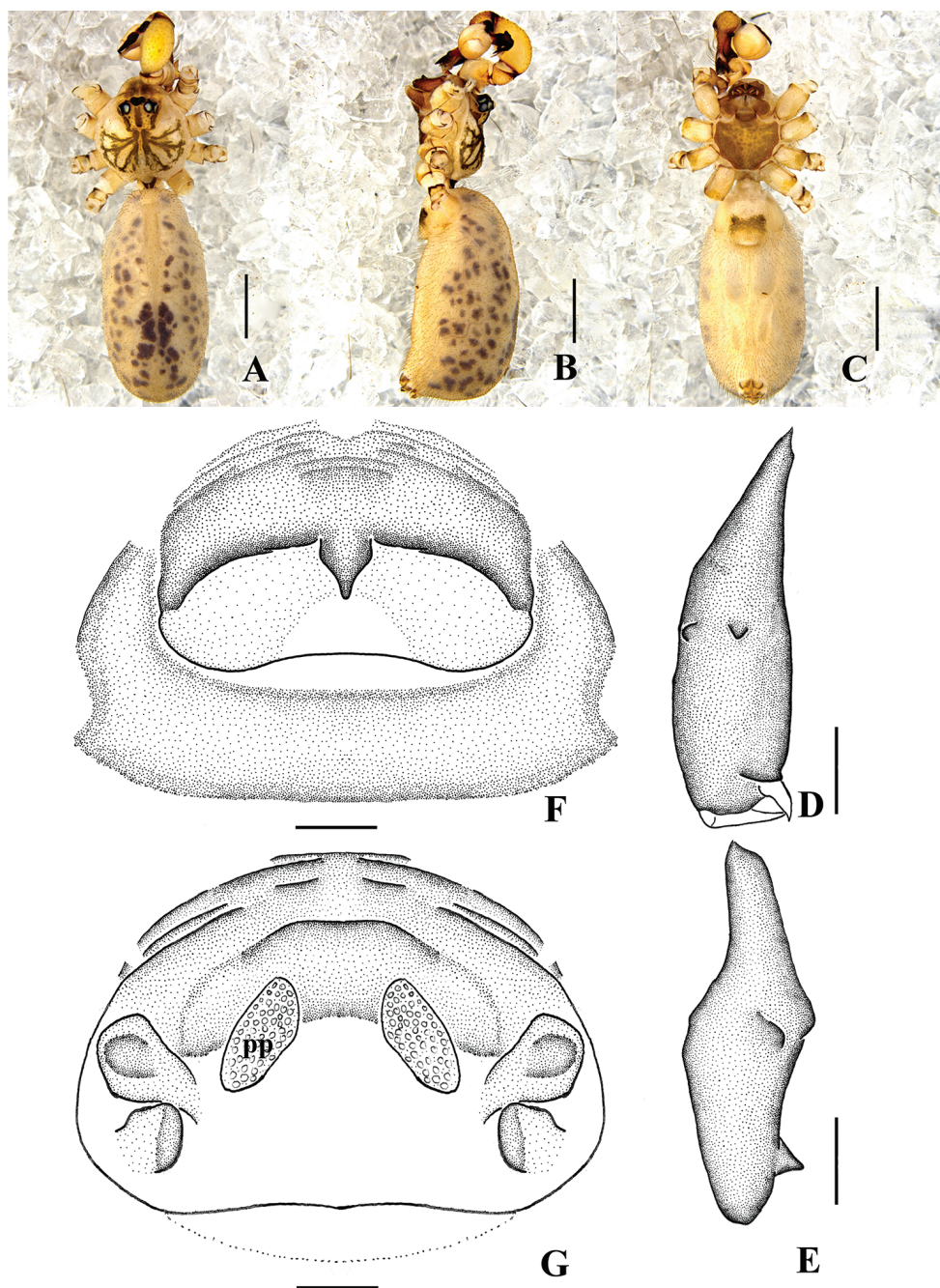


**Figure 2.** *Pholcus papillatus* sp. n., male holotype (**A–D**) and female paratype (**E–F**). **A–B** Bulb and uncus (**A** prolateral view **B** retrolateral view) **C–D** Chelicerae (**C** frontal view **D** lateral view) **E** Epigynum, ventral view **F** Vulva, dorsal view. Scale bars: 0.2 mm.



**Figure 3.** *Pholcus papillatus* sp. n., male holotype. **A–B** Pedipalpus (**A** prolateral view **B** retrolateral view) **C–D** Distal part of procurus (**C** prolateral view **D** retrolateral view). Scale bars: 0.2 mm.





**Figure 4.** *Pholcus papillatus* sp. n., male holotype (A–E) and female paratype (F–G). A–C Habitus (A dorsal view B lateral view C ventral view) D–E Chelicerae (D, frontal view E lateral view) F Epigynum, ventral view G Vulva, dorsal view. Scale bars: 0.2 mm (D–G); 1.0 mm (A–C).



PLE-PLE 0.64. MOA 0.24 long, front width 0.18, back width 0.39. labium wider than long (0.31/0.18). Sternum wider than long (1.00/0.74). Measurements of legs: I 36.60 (9.55 + 0.58 + 9.59 + 15.41 + 1.47), II 26.66 (7.33 + 0.54 + 5.88 + 11.54 + 1.37), III 22.32 (6.51 + 0.58 + 5.38 + 8.58 + 1.27), IV 24.49 (7.01 + 0.52 + 5.72 + 9.88 + 1.36); tibia I L/d: 60. Leg formula: 1243. Epigynum (Figs 2E and 4F) brown, roughly triangular, with distinct patterns and a teat-shaped apophysis on the top. Dorsal view of vulva (Figs 2F and 4G) with a rainbow-shaped, sclerotized arch anteriorly and two long ovoid pore plates, and a nearly meniscate sclerite.

**Variation.** Male: Total body length: 4.58, 4.90. Tibia I (n = 2): 8.96, 9.59 (mean: 9.28). Female: Total body length: 4.58, 4.63, 4.81. Tibia I (n = 3): 9.55, 9.70, 9.78 (mean 9.68).

**Distribution.** Only known from the type locality.

**Remarks.** The females also resemble *P. foliaceus* Peng & Zhang, 2013, but can be distinguished by the precurved margin of anterior plate of the epigynum and the long ovoid pore plates (Figs 2E–F, 4F–G).

### *Pholcus curvus* sp. n.

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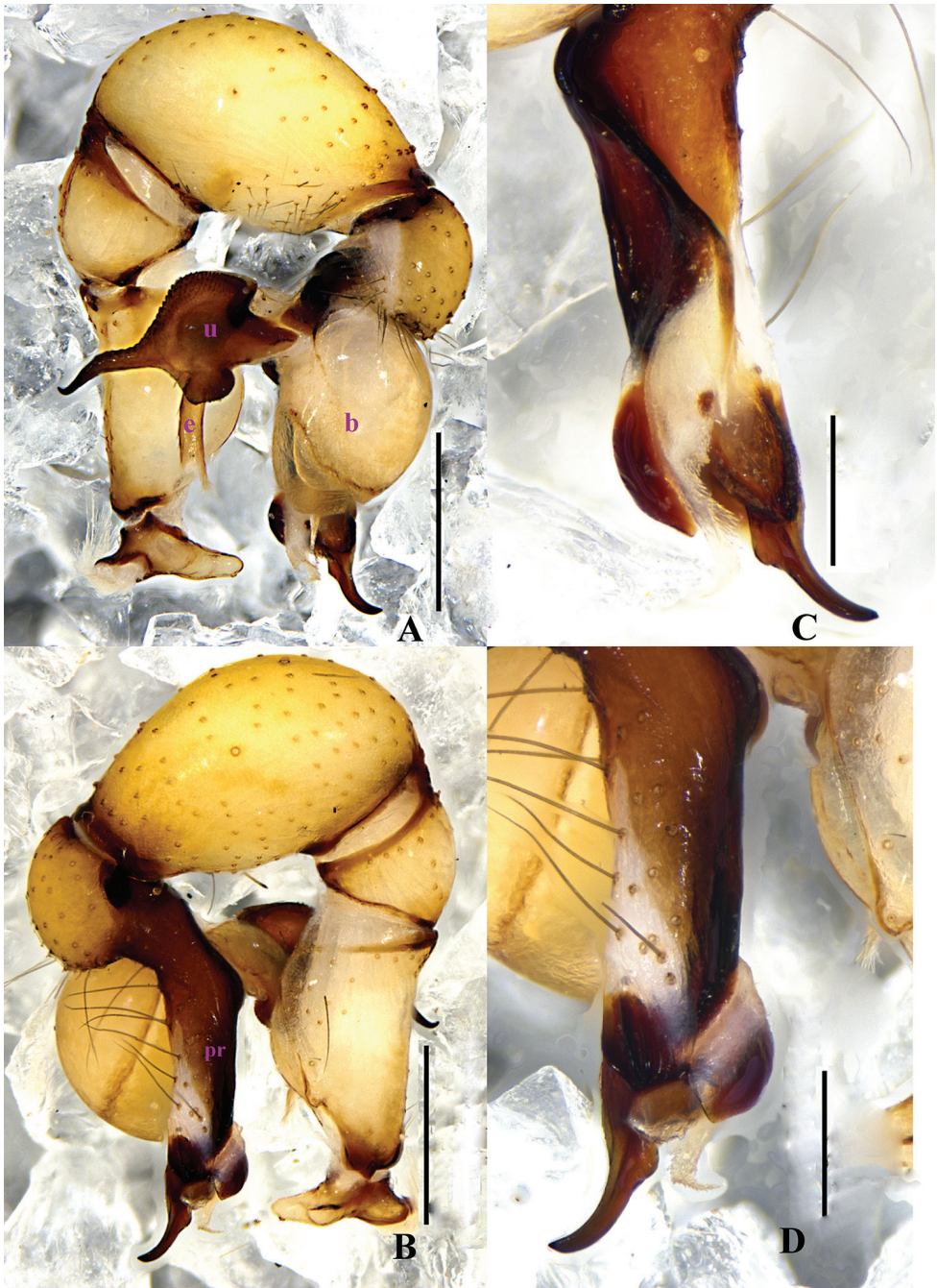
Figs 5–8

**Type material. Holotype:** male (MHBU), CHINA: Hebei Province, Fuping County, Longquanguan Town, Heiyagou Village, 38°16'N, 114°05'E, alt. 900 m, 5 August 2014, B.S. Zhang leg. **Paratypes:** 2 males and 8 females (MHBU), same data as in holotype.

**Etymology.** The specific name is from the Latin word “*curvus*”, in reference to the shape of the palpal uncus; adjective.

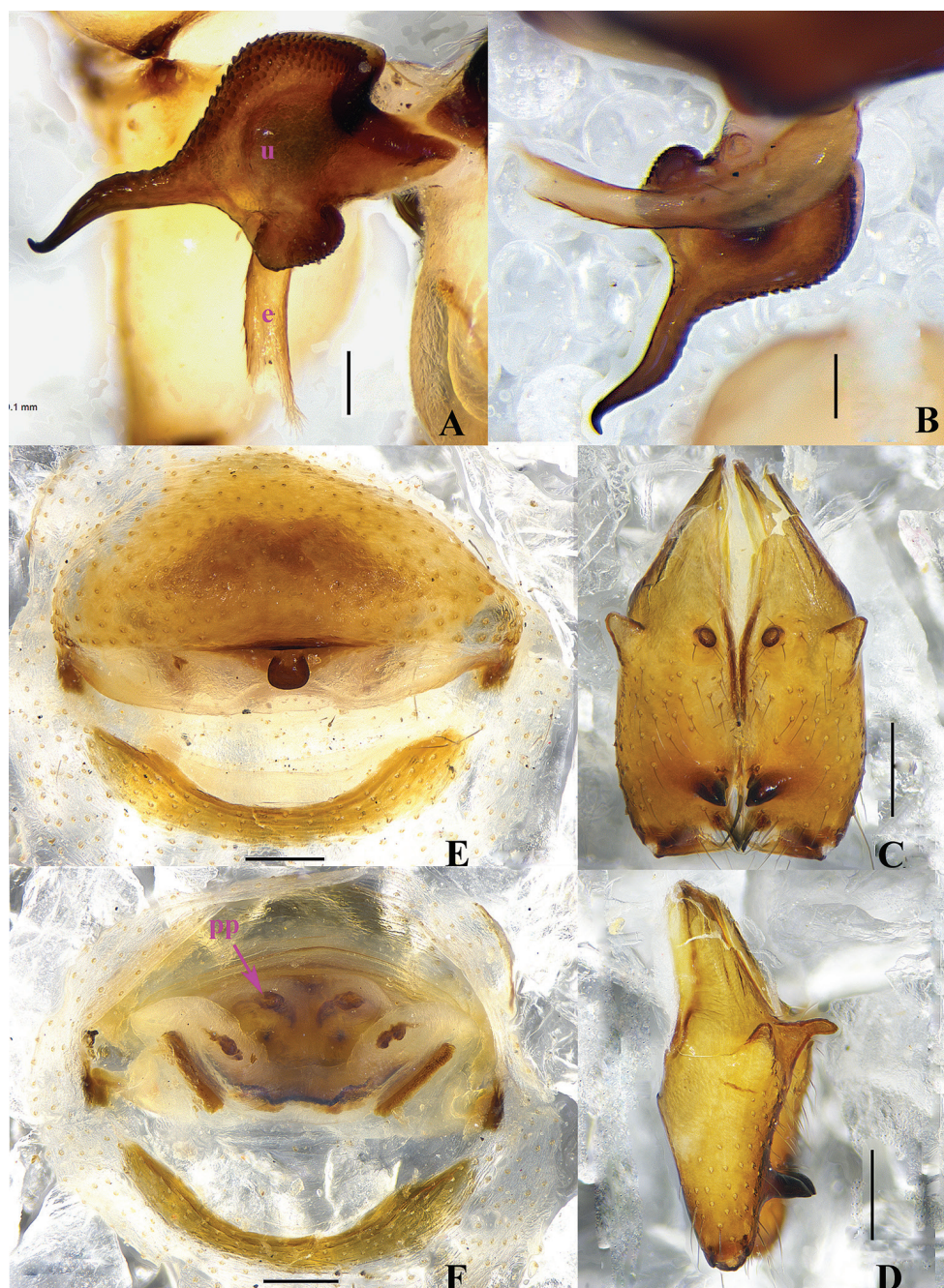
**Diagnosis.** Distinguished by the S-shaped tip of uncus, the long and curve beak-shaped tip of the procurus, the hat-shaped membranous projection near tip, the small and almost quadrate-shaped epigynal apophysis (Figs 5A–D, 7A–D, 6E, 8F).

**Description.** Male (holotype): Total length 4.50 (4.63 with clypeus), prosoma 1.36 long, 1.41 wide, opisthosoma 2.85 long, 1.24 wide. Habitus as in Fig. 8A–C. Dorsal shield of prosoma pale grey, with brown radiated stripes and bands marginally; thoracic groove distinct; ocular area elevated, with short eye-stalks; ocular area yellow-brown, with a median brown band and two lateral brown bands beside PLEs, dorsal prosoma and ocular area both with dispersed brown spots, of them, two distinct spots behind PME; clypeus 0.20 high, brown. Diameter AME 0.07, ALE 0.08, PME 0.15, PLE 0.13. Distance AME-AME 0.06, AME-ALE 0.05, PME-PME 0.24, PME-PLE 0.06, ALE-ALE 0.49, PLE-PLE 0.62. MOA 0.24 long, front width 0.22, back width 0.42. Chelicerae as in Figs 6C–D and 8D–E, with pair of black apophyses, pair of unsclerotized thumb-shaped apophyses proximolaterally, and pair of nearly long finger-shaped apophyses frontally. Labium and endites brown, distal part pale, labium wider than long (0.32/0.17). Sternum wider than long (1.00/0.72), brown, median part of

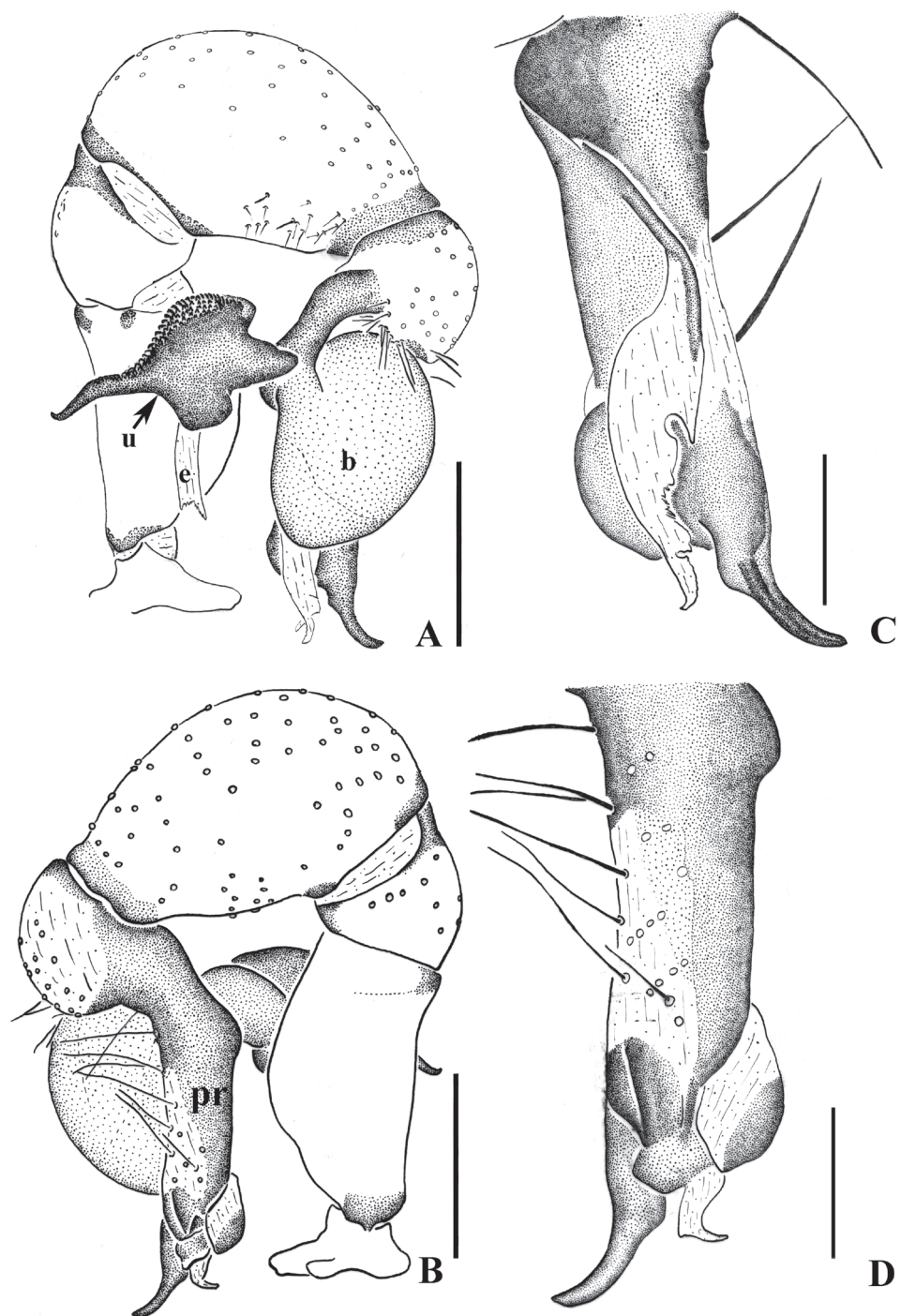


**Figure 5.** *Pholcus curvus* sp. n., male holotype. **A–B** Pedipalpus (**A** prolateral view **B** retrolateral view) **C–D** Distal part of procurus (**C** prolateral view **D** retrolateral view). Scale bars: 0.2 mm.



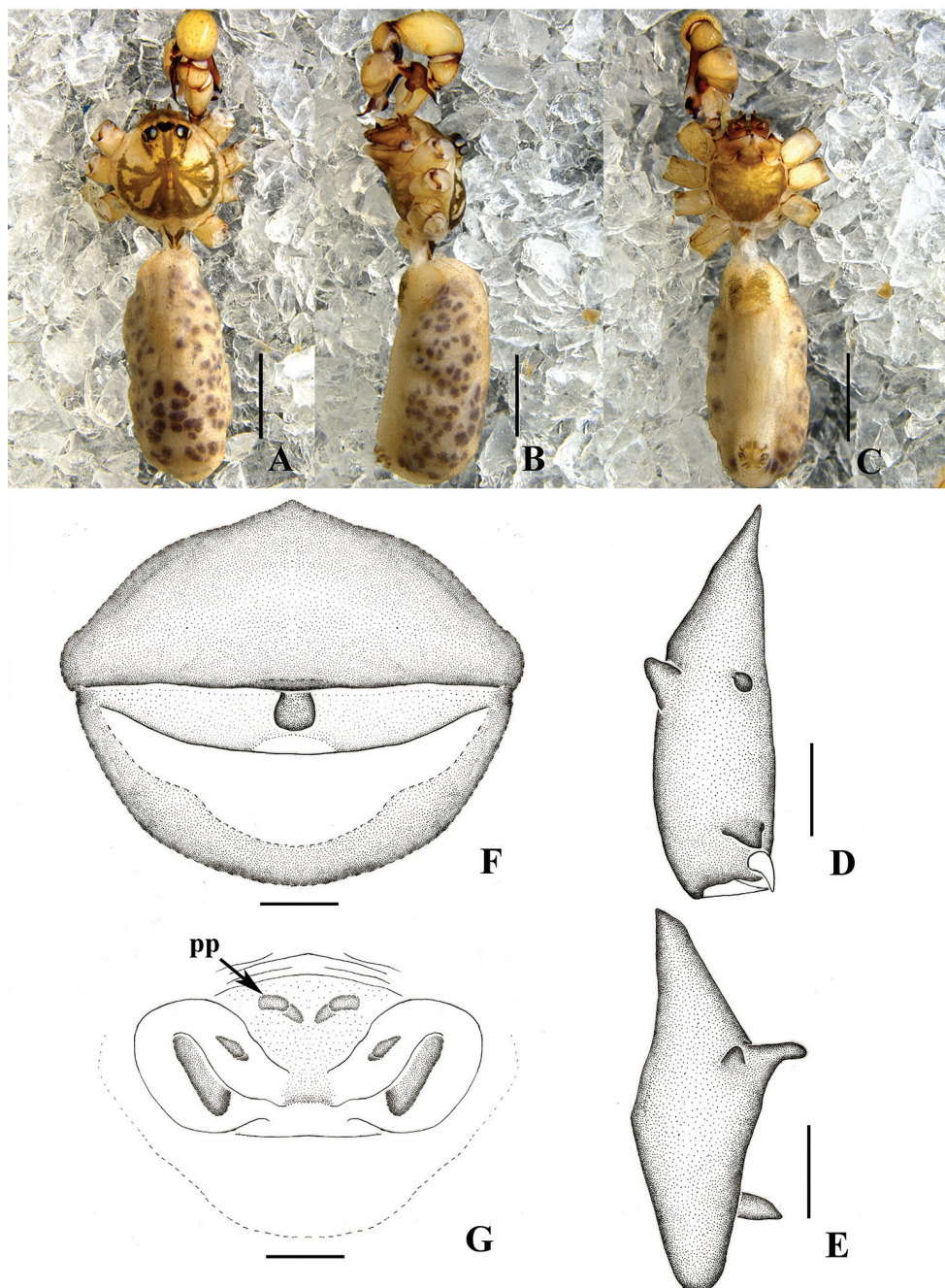


**Figure 6.** *Pholcus curvus* sp. n., male holotype (A–D) and female paratype (E–F). A–B Bulb and uncus (A prolateral view B retrolateral view) C–D Chelicerae (C frontal view D lateral view) E Epigynum, ventral view F Vulva, dorsal view. Scale bars: 0.1 mm (A, B); 0.2 mm (C–F).



**Figure 7.** *Pholcus curvus* sp. n., male holotype. **A–B** Pedipalpus (**A** prolateral view **B** retrolateral view) **C–D** Distal part of procursus (**C** prolateral view **D** retrolateral view). Scale bars: 0.2 mm.





**Figure 8.** *Pholcus curvus* sp. n., male holotype (A–E) and female paratype (F–G). A–C Habitus (A dorsal view B lateral view C ventral view) D–E Chelicerae (D frontal view E lateral view) F Epigynum, ventral view G Vulva, dorsal view. Scale bars: 0.2 mm (D–G); 1.0 mm (A–C).

sternum light. Legs long, brown, with two dark brown ring spots on proximal parts of femora and four dark brown ring spots on tibiae. Measurements of legs: I 33.98 (9.57 + 0.53 + 9.23 + 13.23 + 1.42), II 24.63 (6.96 + 0.58 + 6.30 + 9.49 + 1.30), III 17.75 (5.22 + 0.41 + 4.32 + 6.79 + 1.01), IV 23.52 (6.90 + 0.47 + 5.89 + 9.16 + 1.10); tibia I L/d: 60. Leg formula: 1243. Opisthosoma pale grey, with dark spots dorsally and laterally. Pedipalpi as in Figs 5A–D and 7A–D; trochanter with a moderate ventral apophysis; proximal femur with a small apophysis retrolaterally; tibia with a sheet-shaped projection prolaterally; procursus simple and tip with a narrow process, dorsal spines present; uncus long and bent, with a scaly edge; embolus weakly sclerotized.

**Female.** Generally similar to the male. One specimens measured: total length 5.09 (5.26 with clypeus), prosoma 1.27 long, 1.52 wide, opisthosoma 3.59 long, 1.61 wide, clypeus 0.20 high. Diameter AME 0.08, ALE 0.10, PME 0.13, PLE 0.12. Distance AME-AME 0.07, AME-ALE 0.07, PME-PME 0.17, PME-PL 0.05, ALE-ALE 0.45, PLE-PL 0.59. MOA 0.25 long, front width 0.14, back width 0.36. Labium wider than long (0.31/0.17). Sternum wider than long (1.05/0.79). Measurements of legs: I 32.15 (7.99 + 0.55 + 8.09 + 13.42 + 2.10), II 20.24 (6.31 + 0.42 + 5.60 + 6.55 + 1.36), III 17.40 (4.71 + 0.55 + 3.87 + 6.95 + 1.32), IV 20.12 (6.03 + 0.64 + 5.53 + 6.55 + 1.37); tibia I L/d: 50. Leg formula: 1243. Epigynum (Figs 6E and 8F) brown, roughly striped with distinct patterns and an almost quadrate-shaped apophysis on the top. Dorsal view of vulva (Figs 6F and 8G) with a rainbow-shaped, sclerotized arch anteriorly, two bent pore plates, and two long and bent sclerites.

**Variation.** Male: Total body length: 4.38, 4.43, 4.50. Tibia I (n = 3): 9.36, 9.46, 9.57 (mean: 9.46). Female: Total body length 4.78–5.09. Tibia I (n = 8): 7.65–7.99 (mean 7.81).

**Distribution.** Only known from the type locality.

**Remarks.** Among the *P. phungiformes* group, the males of the new species resemble *P. hamatus* Tong & Ji, 2010 by the following: uncus with a narrow, long and bent tip and a robust apophysis, without appendix and pseudo-appendix (Figs 5A, 6A–B, 7A). The females of the new species are distinguished from those of *P. hamatus* by the small and almost quadrate-shaped epigynal apophysis (Figs 6E, 8F).

### *Pholcus auricularis* sp. n.

<http://zoobank.org/294FFD7E-3826-48F3-8E79-A5BC1FA40825>

Figs 9–12

**Type material. Holotype:** male (MHBU), CHINA: Hebei Province, Fuping County, Longquanguan Town, Liaodaobei Village, 38°16'N, 114°17'E, alt. 1050 m, 5 August 2014, B.S. Zhang leg. **Paratypes:** 6 females (MHBU), same data as in holotype.

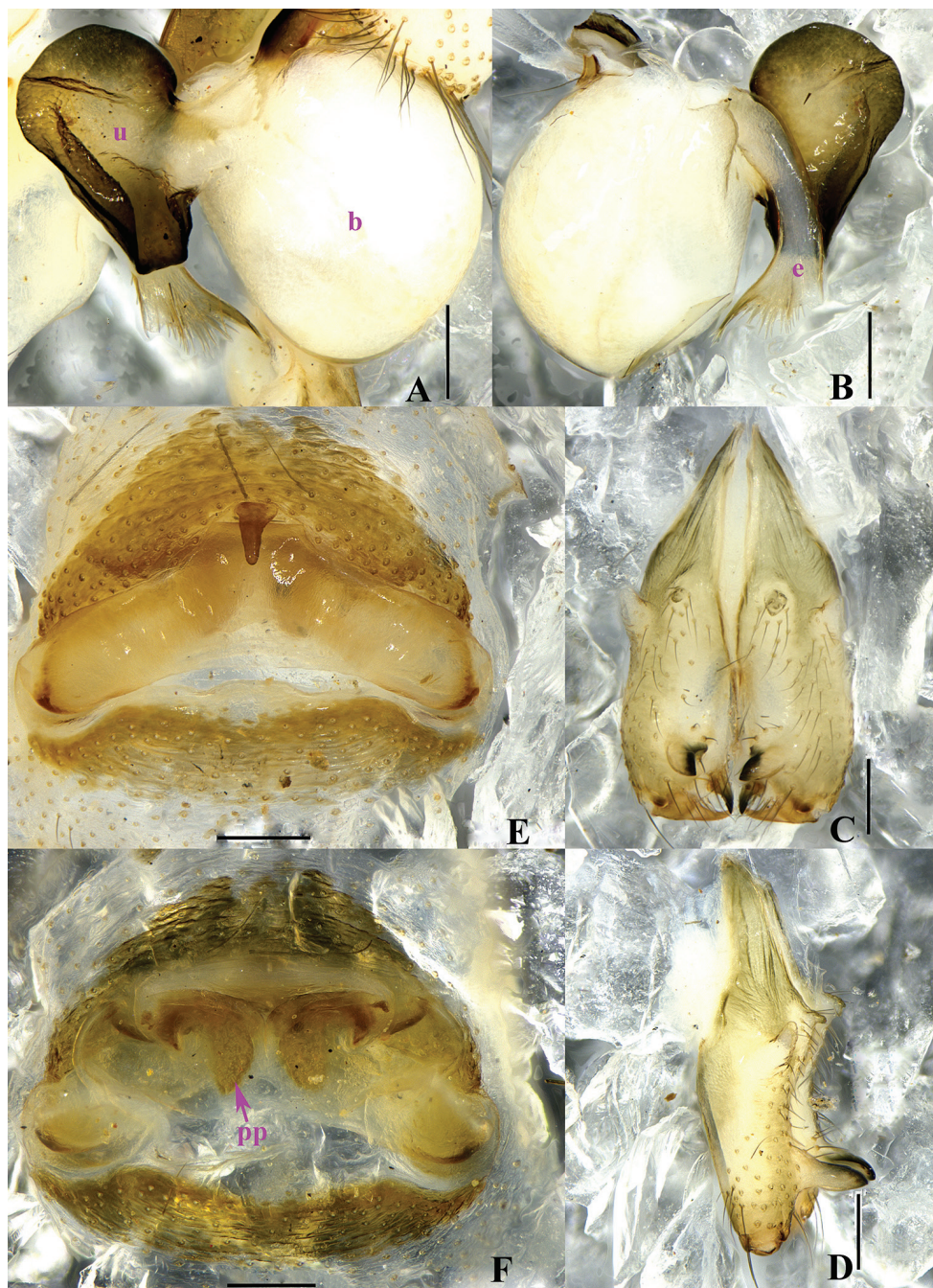
**Etymology.** The specific name is from the Latin word “*auricularis*”, in reference to the shape of pedipalpi; adjective.



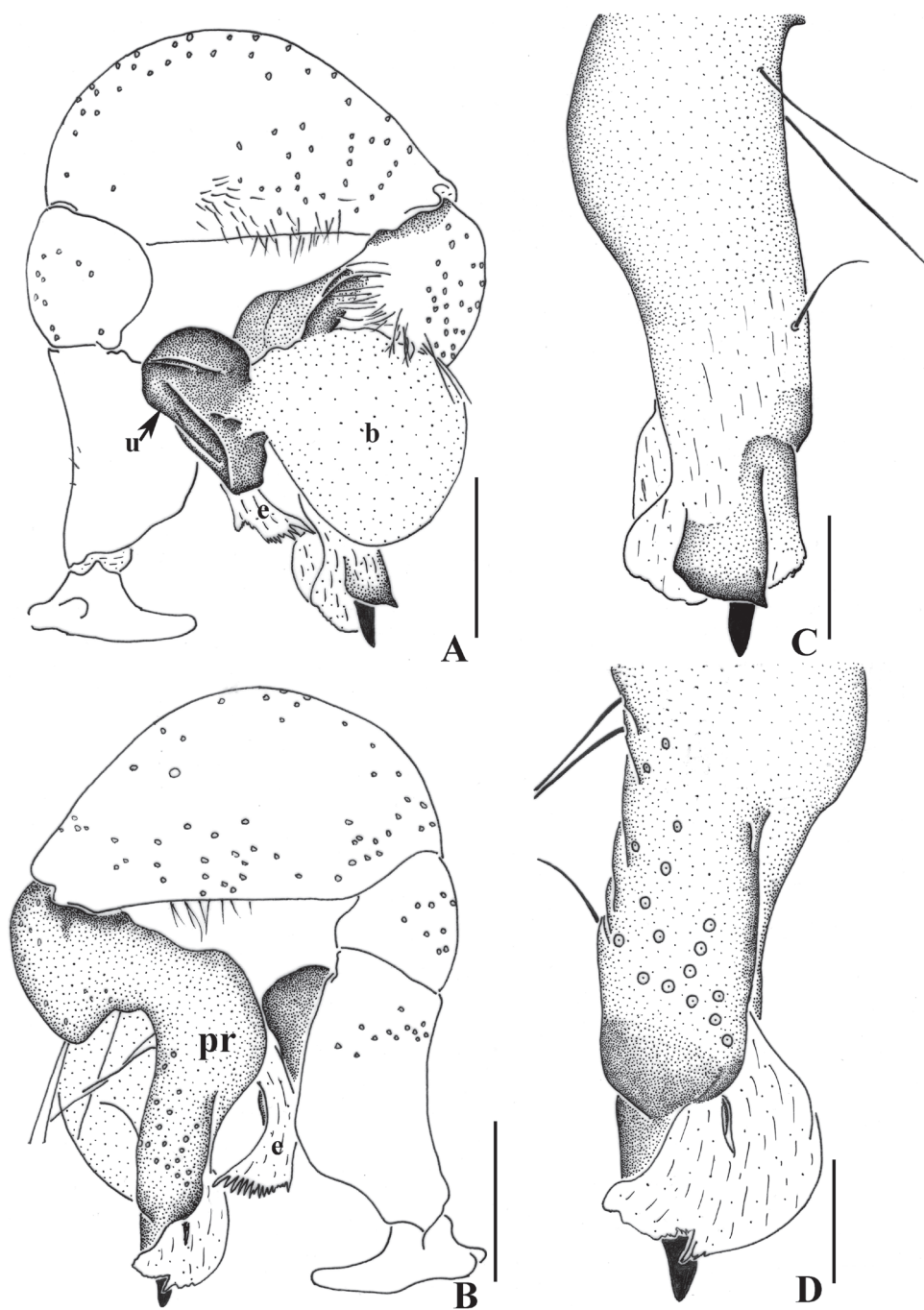


**Figure 9.** *Pholcus auricularis* sp. n., male holotype. **A–B** Pedipalpus (**A** prolateral view **B** retrolateral view) **C–D** Distal part of procurus (**C** prolateral view **D** retrolateral view). Scale bars: 0.2 mm (**C, D**); 0.5 mm (**A, B**).



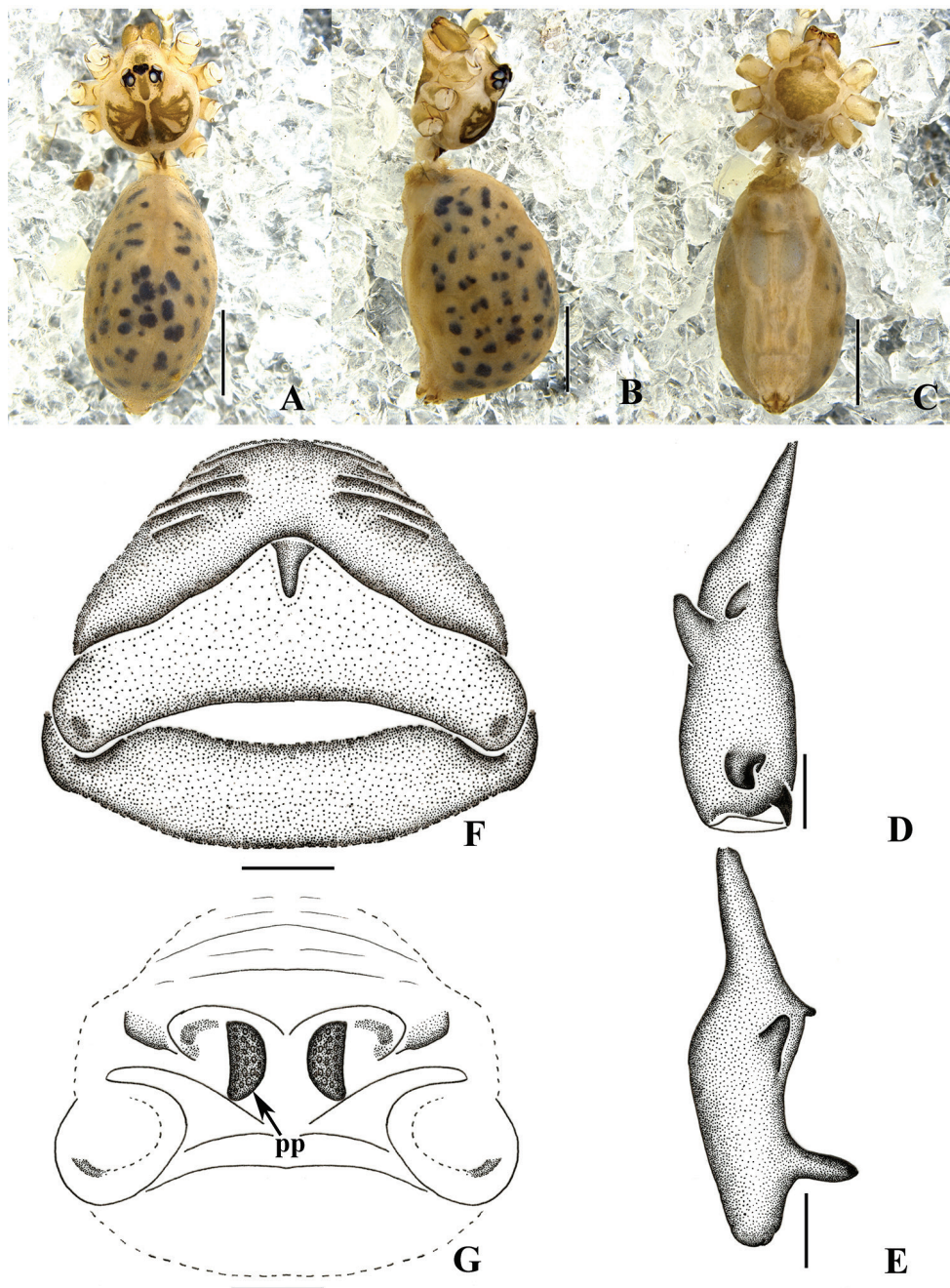


**Figure 10.** *Pholcus auricularis* sp. n., male holotype (A–D) and female paratype (E–F) A–B Bulb and uncus (A prolateral view B retrolateral view) C–D Chelicerae (C frontal view D lateral view) E Epigynum, ventral view F Vulva, dorsal view. Scale bars: 0.2 mm.



**Figure 11.** *Pholcus auricularis* sp. n., male holotype. **A–B** Pedipalpus (**A** prolateral view **B** retrolateral view) **C–D** Distal part of procurus (**C** prolateral view **D** retrolateral view). Scale bars: 0.2 mm (**C, D**); 0.5 mm (**A, B**).





**Figure 12.** *Pholcus auricularis* sp. n., male holotype (A–E) and female paratype (F–G) A–C Habitus (A dorsal view B lateral view C ventral view) D–E Chelicerae (D frontal view E lateral view) F Epigynum, ventral view G Vulva, dorsal view. Scale bars: 0.2 mm (D–G); 1.0 mm (A–C).

**Diagnosis.** Distinguished by the combination of the following characters: uncus thin, ear-shaped, the tip of procurus thin and extending downward, epigynal apophysis short, thin and clavate, the tip thinner (Figs 9A–D, 10A–B, 10E, 11A–D, 12F).

**Description.** Male (holotype): Total length 4.13 (5.32 with clypeus), prosoma 1.07 long, 1.19 wide, opisthosoma 2.87 long, 1.54 wide. Habitus as in Figs 12A–C. Dorsal shield of prosoma pale grey, with brown radiated stripes and bands marginally; thoracic groove absent; ocular area elevated, with short eye-stalks; ocular area pale grey, with a wide median brown band and two lateral brown bands beside PLEs, dorsal prosoma and ocular area both with dispersed dark brown spots, of them, two distinct spots behind PME; clypeus 0.21 high, pale grey, median part with brown patch. Diameter AME 0.07, ALE 0.10, PME 0.08, PLE 0.09. Distance AME-AME 0.04, AME-ALE 0.06, PME-PME 0.17, PME-PL 0.06, ALE-ALE 0.47, PLE-PL 0.54. MOA 0.24 long, front width 0.16, back width 0.35. Chelicerae as in Figs 10C–D and 12D–E, with pair of long apophyses distally and prolateral part black, pair of unsclerotized thumb-shaped apophyses proximolaterally, and pair of nearly nipple-shaped apophyses frontally. Labium and endites brown, distal part pale, labium wider than long (0.27/0.19). Sternum wider than long (0.73/0.58), brown, median part of sternum light, margin pale grey. Legs long, pale grey, with four brown ring spots on femora and tibiae, one brown ring spot on proximal parts of metatarsi. Measurements of legs: I 21.14 (5.61 + 0.44 + 5.61 + 7.87 + 1.61), II 15.13 (4.54 + 0.27 + 3.75 + 5.34 + 1.23), III 11.14 (3.15 + 0.34 + 2.72 + 3.90 + 1.03), IV 14.89 (4.22 + 0.30 + 3.69 + 5.28 + 1.40); tibia I L/d: 51. Leg formula: 1243. Opisthosoma pale grey, with dark spots dorsally and laterally. Pedipalpi as in Figs 9A–D and 11A–D; trochanter with a moderate ventral apophysis; tibia with a sheet-shaped projection prolaterally; procurus simple, its tip extending downward, dorsal spines absent; uncus and embolus weakly sclerotized.

**Female.** Similar to male. One specimen measured: total length 4.95 (5.12 with clypeus), prosoma 1.35 long, 1.48 wide, opisthosoma 3.42 long, 2.35 wide. clypeus 0.22 high, yellow. Diameter AME 0.04, ALE 0.10, PME 0.10, PLE 0.12. Distance AME-AME 0.07, AME-ALE 0.06, PME-PME 0.23, PME-PL 0.06, ALE-ALE 0.50, PLE-PL 0.61. MOA 0.21 long, front width 0.19, back width 0.37. Labium wider than long (0.27/0.18). Sternum wider than long (0.97/0.75). Measurements of legs: I 21.82 (7.00 + 0.56 + 5.59 + 7.42 + 1.25), II 18.03 (5.23 + 0.55 + 4.57 + 6.53 + 1.15), III 13.44 (3.80 + 0.39 + 3.31 + 4.91 + 1.03), IV 17.74 (4.86 + 0.52 + 4.48 + 6.69 + 1.19); tibia I L/d: 63. Leg formula: 1243. Epigynum (Figs 10E and 12F) brown, roughly triangular, with distinct patterns and a short rod-like apophysis on the top. Dorsal view of vulva (Figs 10F and 12G) with an M-shaped, sclerotized arch anteriorly, two closely spaced semilunar pore plates.

**Variation.** Female: Total body length 4.68–4.95. Tibia I (n = 6): 6.56–7.00 (mean 6.86).

**Distribution.** Only known from the type locality.

**Remarks.** Among the *P. phungiformes* group, the new species resembles *P. allocospilus* Zhu & Gong, 1991 and *P. fengchen* Zhang & Zhu, 2009, but can be distinguished from those by: the short and thick club-shaped projection on tip of procurus, chitinized plate of the epigynum triangular arch (Figs 9C–D, 10E, 11C–D, 12F).



## Conclusions

The *P. phungiformes* group includes 52 nominal species, of which 33 species were recorded from China (Zhang and Zhu 2009; Tong and Ji 2010; Tong and Li 2010; Huber 2011b; Chen, Zhang and Zhu 2011; Yao and Li 2012; Peng and Zhang 2013; Seo 2014; Liu and Tong 2015). Most of these Chinese species are distributed in northeastern China: from Hebei Province *P. alloctospilus*, *P. pennatus*, *P. zhuolu*, *P. triangulatus*, *P. wangxidong*, *P. chicheng*, *P. datan*, *P. babao*, *P. wuling*, *P. jinniu*, *P. exilis*, *P. papillatus* sp. n., *P. curvus* sp. n. and *P. auricularis* sp. n. are known, from Beijing municipality *P. beijingensis*, *P. brevis*, from Liaoning Province *P. suizhongicus*, *P. jiuwei*, *P. fengcheng*, *P. phoenixus*, *P. gaoi*, *P. wangtian*, *P. tongi*, *P. wangi*, *P. decorus*, *P. hamatus*, *P. lingulatus*, *P. foliaceus*, *P. xianrendong* and *P. sublingulatus*, from Shanxi Province *P. luya*, and from both Hebei and Liaoning Provinces *P. clavimaculatus*, only *P. xingren* occurs in Guizhou Province.

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# A preliminary synopsis on amber scorpions with special reference to Burmite species: an extraordinary development of our knowledge in only 20 years

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## Abstract

A preliminary study on fossil scorpions found in amber, from the Lower Cretaceous through the Palaeocene and up to the Miocene is proposed. Scorpions remain rare among the arthropods found trapped in amber. Only 24 specimens are known from Cretaceous amber, representing eight families and subfamilies, ten genera and 21 species; in parallel, 10 specimens have been recorded from Baltic amber representing seven genera and ten species. A few more recent fossils from Dominican and Mexican amber have also been described. The present study of a new scorpion specimen from the Cretaceous amber of Myanmar (Burmite) resulted in the description of one new species, *Betaburmesebuthus bellus* sp. n. – belonging to the subfamily Palaeoburmesebuthinae Lourenço, 2015. The new description brings further elements to the clarification of the status of this subfamily, which is now raised to family level. Once again, this new Burmite element attests to the considerable degree of diversity in the Burmese amber-producing forests.

## Keywords

Burmite, Cretaceous, fossil, Myanmar, new species, Palaeoburmesebuthidae, scorpion

## Introduction

Among the fossil arthropods found in amber, scorpions remain extremely rare. The renewal of studies on scorpions trapped in amber began in the early 1980s when a few specimens from Dominican and Mexican amber were described (Schawaller 1979, 1982; Santiago-Blay and Poinar Jr. 1988, 1993; Santiago-Blay et al. 1990). Even if some new taxa from Dominican and Mexican amber are yet to be described, the amber fossils found in these regions of the world seem in all cases closely related to the extant scorpion taxa of the Caribbean and North/Central America.

Baltic amber was the first to provide fossil scorpions, at the beginning of the 19<sup>th</sup> century. The first described species was *Scorpio schweiggeri* (Holl 1829). However, both the description and the illustration of this species lack accuracy; the only conclusion that can be reached is that the scorpion most certainly belongs to the family Buthidae C. L. Koch, 1837. This species has been ignored by most authors, although Schawaller (1979) published a brief comment in which he suggested that *S. schweiggeri* should be considered a *nomen nudum*. Since the type-specimen was lost, there is not much that can be added on its status.

A second species, *Tityus eogenus* Menge, 1869, was also described. Unlike *Scorpio schweiggeri*, *Tityus eogenus* has received the attention of many authors, first because of its assignment by Menge to a typically Neotropical extant genus, and secondly because the type-specimen was apparently lost soon after its description, which prevented the confirmation of its taxonomic position. Therefore this Baltic amber scorpion has turned into a kind of curiosity. Because of the early disappearance of Menge's material, for more than one hundred years this Baltic amber fossil has been the subject of discussion and speculation, and was mentioned in a number of publications (e. g. Werner 1935; Petrunkevitch 1955, 1958; Larsson 1978; Schawaller 1979; Kjellesvig-Waering 1986; Spahr 1993). Menge's collection included two specimens, but apparently only one was preserved well enough to be of scientific value (Menge 1869; Larsson 1978). Based on the characters supplied by Menge (1869), it can only be concluded that *Tityus eogenus* is indeed a buthid scorpion. It could, however, be assigned equally well to any of several genera within this family.

In 1995, a new specimen of scorpion from Baltic amber was located in Hamburg, Germany. After the examination of all visible characters it was determined as a member of the family Buthidae, belonging to a new genus and a new species, allied to the genus *Lychas* C. L. Koch, 1845. Nothing, however, could clearly associate this specimen to the two species previously described by Holl (1829) and Menge (1869). The main findings of these studies demonstrated that this Baltic amber scorpion could be associated with the Old World extant fauna. Several new discoveries followed and were subject of a number of studies since 1996 (Lourenço 2004, 2012a; Lourenço and Weitschat 1996, 2000, 2001, 2005, 2009; Lourenço et al. 2005). This led to discovering and describing a total of ten specimens representing seven new genera and ten new species, globally confirming the relationships of this extinct fauna with the elements of the extant buthid fauna yet present in both the old and new worlds.



Even more relevant was the description, in the last 15 years, of 24 specimens from Cretaceous amber, representing eight families and subfamilies, ten genera and 21 species. These fossil scorpions trapped in Cretaceous amber can be dated from 135 to 90 Ma. Although several of these elements can be associated with buthoids, such as *Archaeobuthus estephani* Lourenço, 2001 (family Archaeobuthidae Lourenço, 2001) from amber of Lebanon and the several species of the genera *Palaeoburmesebuthus* Lourenço, 2002 and *Betaburmesebuthus* Lourenço, 2015 (subfamily Palaeoburmesebuthinae Lourenço, 2015) both from Burmite amber from Myanmar (Lourenço and Beigel 2015), a number of non-buthoid elements have also been recorded and described. These comprise *Palaeoeuscorpius gallicus* Lourenço, 2003 (family Palaeoeuscorpiidae Lourenço, 2003) from amber of France and several elements from Burmite, namely: *Electrochaerilus buckleyi* Santiago-Blay, Fet, Sölegård & Anderson, 2004 (family Chaerilidae Pocock, 1893), several species of the genus *Chaerilobuthus* Lourenço & Beigel, 2011 (family Chaerilobuthidae Lourenço & Beigel, 2011), *Palaeotrilineatus ellenbergeri* Lourenço, 2012 (family Palaeotrilineatidae Lourenço, 2012), *Archaeoscorpiops cretacicus* Lourenço, 2015 and *Burmesescorpiops groehni* Lourenço, 2016 (subfamily Archaeoscorpiopinae Lourenço, 2015), and finally *Sucinlourencous adrianae* Rossi, 2015 (family Sucinlourencoidae Rossi, 2015). Dated at almost 135 Ma, *Archaeobuthus estephani* remains the oldest fossil scorpion ever found in amber (Lourenço 2001, 2002, 2003, 2012b, 2013, 2015a,b,c,d, 2016; Lourenço and Beigel 2011; Lourenço and Velten 2015, 2016; Rossi 2015; Santiago-Blay et al. 2004).

After the clarification of the familial status of the genus *Palaeoburmesebuthus* (and consequently of the genus *Betaburmesebuthus*) and its placement in the subfamily Palaeoburmesebuthinae, this last subfamily was temporarily accommodated in the family Archaeobuthidae Lourenço, 2001, both because of their association to the buthoid lineage, but in particular because of their location in a similar geological horizon. Nevertheless, the recent study of several almost perfectly preserved specimens, clearly attests their relationship to the buthoids (Lourenço 2015b, c; Lourenço and Velten 2016; this study), in particular based on their trichobothrial patterns, which are almost identical to those of several extant buthoids. Based on these new characters (see description), the subfamily Palaeoburmesebuthinae is now raised to the familial level as Palaeoburmesebuthidae and placed in the superfamily Buthoidea.

### **The controversial opinions of different authors concerning the presence of buthoid lineages during Mesozoic times**

The exclusion of the family Archaeobuthidae from the buthoids has been proposed by a number of authors (Baptista et al. 2006; Riquelme et al. 2015), mainly on the basis of theoretical speculation. It is well known that both higher classification of scorpion in general and the classification of fossils in particular are controversial issues, which have been largely debated within scorpion taxonomy, especially during the last 20

years. There is no full evidence allowing the inclusion or the exclusion of a given fossil to the Buthoidea lineage.

This new procedure is becoming a common method used by many authors in their attempt to gain credit on the backs of other people's discoveries. In a few cases only the originally described material has been re-examined, but in many other cases the critics were based solely on the originally published descriptions (see Riquelme et al. 2015). In fact, when dealing with systematic studies of a given taxonomic group, it should be a standard practice to refer to and consider the available material cited in previous publications. Moreover, since the available fossil specimens are extremely rare, this paucity of information makes the re-evaluation even more challenging; consequently, it remains unclear to what extent the re-evaluations on the same case specimens are of any value.

In the case of *Archaeobuthus estephani* Lourenço, 2001 (family Archaeobuthidae) all available data are based only on a single but incomplete specimen. The validity of the family Archaeobuthidae was not questioned of itself, but authors such as Baptista et al. (2006) clearly rejected its association to the buthoids. Nevertheless, the available data to date for this unique Lebanon amber fossil is still insufficient to proceed with a revision of the position of this taxon. Therefore, a final decision should await until more information becomes available.

In a recent publication, eight authors have described a new fossil scorpion from Chiapas amber (Riquelme et al. 2015). If this new fossil is considered as rare and outstanding by its authors, it should be noted that not only is Chiapas amber of recent Miocene age, some 20–15 Ma, but also that other species have been recently described from this same type of amber (Lourenço 2014), bringing as much, if not more, morphological information about these fossils. In the case of the species described by Riquelme et al. (2015), some key morphological information such as the trichobothrial pattern is not clearly defined and in all cases not illustrated, attesting that the described specimen was likely not so unique. The major problem, however, with this publication is the fact that the authors rapidly diverge from the main goal, which should have been the thorough description of the new fossil scorpion, and instead proposed a type of global synopsis on scorpion fossils in amber, including comments on sedimentary fossils and even copal sub-fossils. All these extensive sections presented in part in the introduction, comments, and the phylogenetic discussion are not supported by any original data, but remain solely a compilation of data already available in several original or second-hand publications. Furthermore, the presented compilation, including the phylogenetic analyses, is conducted without any new study of the original material, which most certainly remains totally unknown to these authors. Even more controversial is the attempt to produce a synopsis on amber scorpions, thereby ignoring many of the currently known elements. A number of gaps are clearly visible, in particular concerning Cretaceous Burmite amber scorpions. The most critical mistake, however, is the proposition in a suggested cladogram (cf. figure 10 in Riquelme et al. 2015) of the Burmite genus *Palaeoburmesebuthus* as *incertae sedis*, knowing that the familial status of this genus was already clarified several months before their publication (Lourenço 2015a). See systematic section for further discussion.

## Material and methods

The specimen investigated here is preserved in very clear block of pale yellow amber. Details of the block are supplied together with the description of the specimen. Many characters, and in particular several trichobothria, are visible in this specimen, allowing detailed investigation. Some characters, however, are not totally observable mainly because the specimen suffered a certain degree of dissection process within the resin. The schematic drawings provided here are interpretations of what was observable. Illustrations and measurements were produced with the aid of a Wild M5 stereomicroscope equipped with a drawing tube and an ocular micrometer. Measurements follow Stahnke (1970) and are given in mm. Trichobothrial notations follow Vachon (1974). Trichobothria were definitely recorded only when their bothria (areoles) could be observed. Other trichobothria may be suggested by the presence of transverse hairs.

## Systematic description

### Superfamily Buthoidea C. L. Koch, 1837

### Family Palaeoburmesebuthidae Lourenço, 2015, stat. n.

**Diagnosis for the family.** General morphology shows similarities with several elements of extant buthid scorpions. The following combination of features can be used to diagnose the new family (see also Lourenço 2015a): Carapace not granulated, smooth; anterior margin with a moderately marked median concavity, as observed in some extant buthids. chelicerae with moderately long distal teeth which do not clearly overlap; fixed and movable fingers with one basal and one median tooth. Vesicle very long, with a pear-like shape, resembling those of some extant buthids; with a very long aculeus. Fixed and movable fingers of pedipalp chela with a series of small rounded granules, without any conspicuous accessory granules. Trichobothrial pattern with elements resembling those of extant buthid type A (Vachon 1974); dorsal trichobothria of femur disposed in alpha ( $\alpha$ ) or beta ( $\beta$ ) configurations (see Vachon 1975). Tibial spurs present on legs III and IV.

**Type species.** *Palaeoburmesebuthus grimaldii* Lourenço, 2002

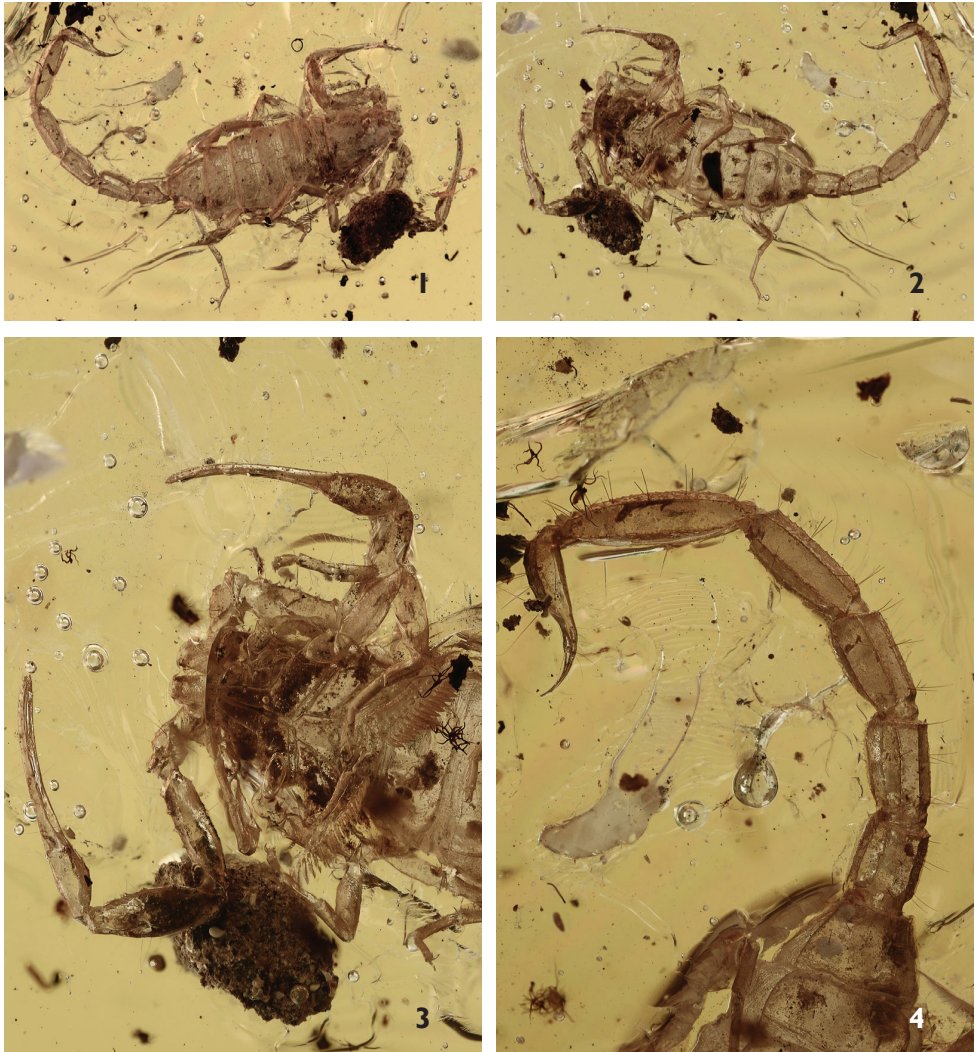
### Genus *Betaburmesebuthus* Lourenço, 2015

#### *Betaburmesebuthus bellus* sp. n.

<http://zoobank.org/21267812-28A4-4D1A-BD96-A2D71ACC11F8>

Figures 1–10

**Holotype.** A juvenile, most certainly a male. Very clear block of pale yellow amber that measured  $21.5 \times 17.0 \times 2$  mm. Only a few inclusions and bubbles prevent a 100%



**Figures 1–4.** *Betaburmesebuthus bellus* sp. n. Male holotype. **1–2** Habitus, dorsal and ventral aspects **3** Ventral aspect in detail, showing sternum, genital operculum and pectines **4** Tergites VI–VII, metasomal segments and telson, dorso-lateral aspect.

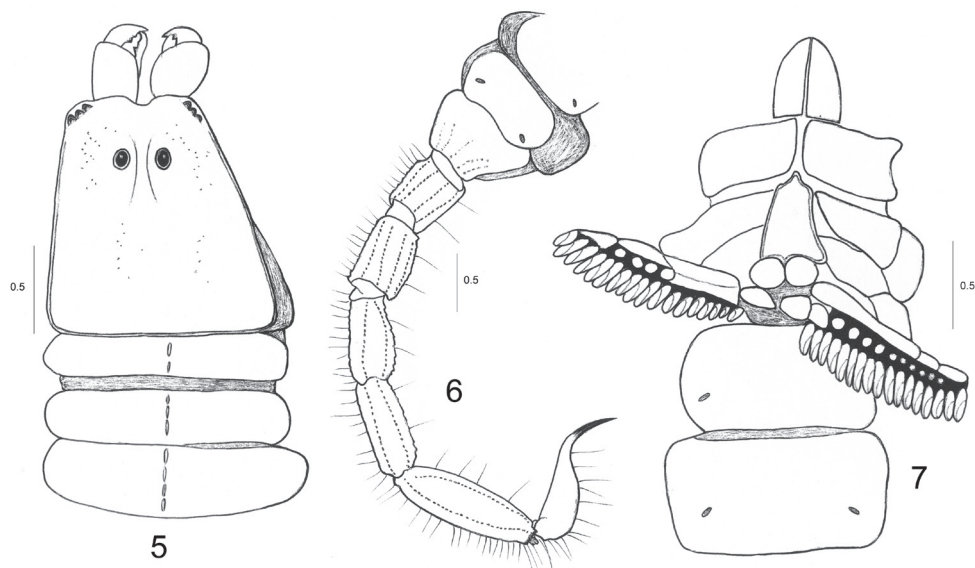
observation of all characters. Type locality and horizon: Myanmar (Burma), Kachin; precise locality unknown; Lower Cretaceous.

**Etymology.** The specific name is an epythet from Latin “*bellus*” meaning beautiful, elegant.

**Repository.** The type specimen is deposited in the collection of the Museum of the Geological-Palaeontological Institut of the University of Hamburg (CeNak - Centrum of Natural History), Coll. N° 4586, Coll. Gröhn N° 11086.

**Diagnosis.** General morphology is globally similar with other species of the genus *Betaburmesebuthus* Lourenço, 2015 and also with extant buthoid scorpions. The



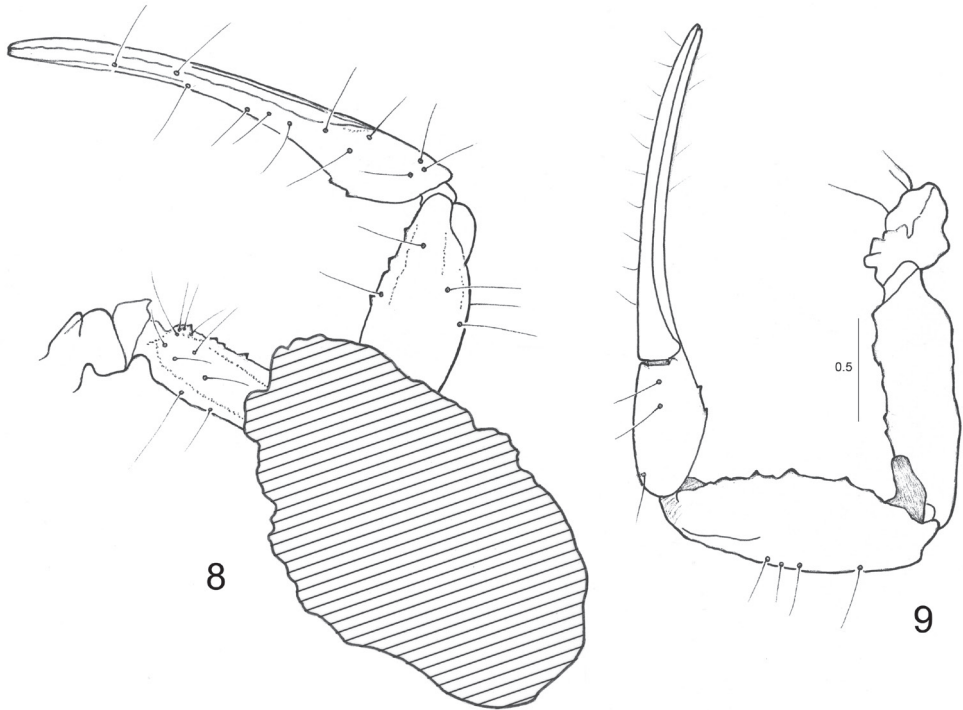


**Figures 5–7.** *Betaburmesebuthus bellus* sp. n. Male holotype. **5** Chelicera, carapace and tergites I–III, dorsal aspect **6** Sternites V–VII showing carinae and spiracles and metasomal segments I–V and telson, ventro-lateral aspect **7** Ventral aspect, showing Coxapophysis, sternum, genital operculum, pectines and sternites with spiracles. Scale bars: 0.5 mm.

following combination of features can be used to diagnose the new species: Carapace weakly to moderately granulated; anterior margin with a moderately marked median concavity. Sternum subpentagonal. Tergites with three carinae, the lateral totally inconspicuous. Sternites with small oval to slit-like spiracle. Metasomal segments I and II with ten carinae; setation on all metasomal segments strongly marked. Fixed and movable fingers of pedipalp chela with a series of small rounded granules and no conspicuous spinoid accessory granules. Trichobothrial pattern with elements resembling those of extant buthid type A (Vachon 1974): at least 1–3 internal, five dorsal, and two external trichobothria on the femur; dorsal trichobothria disposed in beta ( $\beta$ ) configuration (Vachon 1975); one internal and five dorsal trichobothria on patella; 6–7 external trichobothria can be suggested on patella by the presence of fine setae; some are displaced on ventral aspect; 4–5 dorso-external and two ventral on chelal hand; six on fixed finger. Tibial spurs present on legs III and IV.

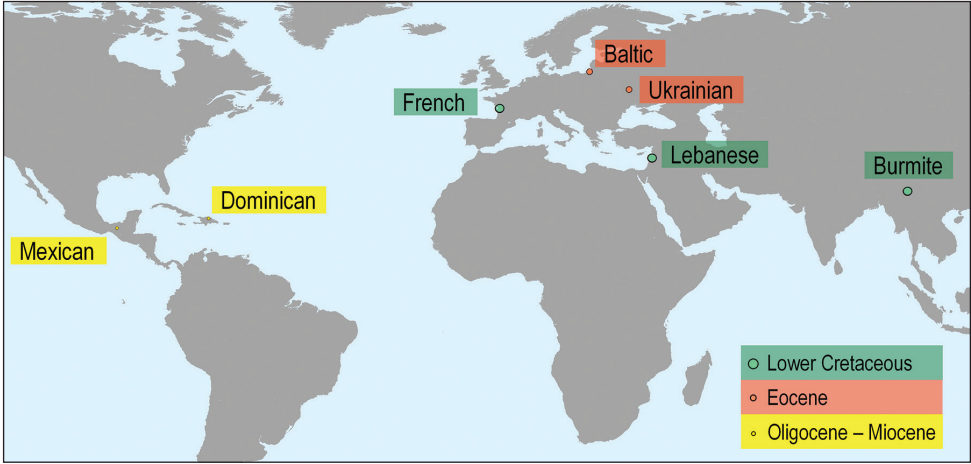
**Description.** Coloration: the scorpion is yellow to slightly reddish-yellow; carapace and tergites yellow; metasomal segments yellow; telson reddish-yellow; pedipalps and legs yellow. Ventral aspect yellow; coxapophysis slightly darker than dorsal aspect.

**Morphology.** Carapace weakly granular; anterior margin with a moderately marked median concavity. Carinae inconspicuous; furrows weak. Median ocular tubercle clearly anterior to the centre of carapace; median eyes moderate in size and separated by more than one ocular diameter. Three pairs of lateral eyes of moderate



**Figures 8–9.** *Betaburmesebuthus bellus* sp. n. Male holotype. Right pedipalp, dorsal and ventral aspects, showing trichobothrial pattern. Dorsal aspect is partially covered by an inclusion. Scale bar: 0.5 mm.

size. Sternum subpentagonal. Mesosomal tergites weakly granular, with one median carina; lateral carinae totally inconspicuous; VII with five weakly marked carinae. Pectines large, with 18–17 teeth; fulcra absent. Sternites weakly granular to smooth, with small oval to slit-like spiracles. Metasomal segment I to IV rounded with 10–10–8–8 carinae; segment V slender with five carinae; dorsal carinae of segments I–IV with minute spinoid granules; dorsal aspect of segments I to V weakly depressed; setation on all segments strongly marked. Telson with a very long pear-shaped vesicle; weakly granular to smooth; aculeus extremely long and moderately curved; setation strongly marked. Cheliceral dentition only partially visible; fixed and movable fingers with one basal tooth observable; distal teeth moderately long (Vachon 1963). Pedipalp femur pentacarinat; patella with 6–7 carinae; internal face of femur and patella with spinoid granules. Chela with weakly marked carinae; all faces not granular, smooth. Fixed and movable fingers each with one series of small rounded granules and no conspicuous spinoid accessory granules; extremity of fingers with stronger spinoid granules; setation of pedipalps weakly marked. Trichobothriotaxy recalling type A (Vachon 1974) of extant buthids: at least 1–3 internal, five dorsal, and two external trichobothria on the femur; dorsal trichobothria disposed in beta ( $\beta$ ) configuration (Vachon 1975); one internal, five dorsal, and no ventral trichobothria on patella; 6–7 external trichobothria can be suggested on patella by the presence of fine setae; some are displaced on the



**Figure 10.** World map showing the sites where scorpions included in amber have been found.

ventral aspect 5-6 dorso-external and two ventral on chelal hand; six on fixed finger. Tibial spurs present on legs III and IV, moderately marked.

Morphometric values (in mm) of male juvenile holotype of *Betaburmesebuthus bellus* sp. n.

Total length 11.80 (including telson). Carapace: length 1.40, anterior width 0.87, posterior width 1.40. Mesosoma length 3.60. Metasomal segments. I: length 0.74, width 0.60; II: length 0.84, width 0.60; III: length 0.94, depth 0.57; IV: length 1.14, depth 0.50; V: length 1.67, depth 0.47. Telson length 1.47. Vesicle: depth 0.37. Pedipalp: femur length 1.14, width 0.34; patella length 1.24, width 0.47; chela length 2.24, width 0.34; movable finger length 1.57.

### Key to the species of Palaeoburmesebuthidae

- 1 Dorsal trichobothria of pedipalp femur in alpha disposition ..... *Palaeoburmesebuthus-2*
- Dorsal trichobothria of pedipalp femur in beta disposition ..... *Betaburmesebuthus-3*
- 2 Telson aculeus moderately long and sharp; metasomal segments III-V with moderately marked carinae ..... *P. grimaldii*
- Telson aculeus extremely long and sharp; metasomal segments III-V with strongly marked carinae ..... *P. ohlboffi*
- 3 Internal face of pedipalp patella without any or to a maximum one spinoid tubercle ..... 4
- Internal face of pedipalp patella with two or four strong spinoid tubercles .... 6
- 4 Spiracles rounded ..... 5
- Spiracles slit-like ..... *B. bellus* sp. n.

- 5      Pectines with 20–20 teeth ..... *B. kobberti*
- Pectines with 14–15 teeth ..... *B. muelleri*
- 6      Internal face of pedipalp patella with two strong spinoid tubercles; two pairs  
of lateral eyes ..... *B. bidentatus*
- Internal face of pedipalp patella with four strong spinoid tubercles; three  
pairs of lateral eyes ..... *B. fleissneri*

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# *Evemphyron sinense*, a new genus and species infesting legume seedpods in China (Coleoptera, Attelabidae, Rhynchitinae)

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## Abstract

A new genus *Evemphyron* Alonso-Zarazaga, Lv & Wang, **gen. n.**, belonging to Attelabidae Rhynchitinae, is described. Its single species, *Evemphyron sinense* Alonso-Zarazaga, Lv & Wang, **sp. n.**, was reared from larvae found inside seed pods of the legume *Callerya dielsiana* (Fabaceae, Millettieae) in Sichuan Province (China). The species is figured and placed in the Deporaini because of the presence of minute labial palpi, the strongly crescentic apex of the postmentum, and the apodemes of male IX sternite and female VIII sternite curved sinistro-anterially near their cephalic end. It shows 3-segmented labial palpi and male sex patches on the procoxae, characters that suggest a basal position in the tribe.

## Keywords

Attelabidae, *Callerya dielsiana*, Deporaini, east Palaearctic, *Eusproda*, Fabaceae, legume, new genus, new species, Rhynchitinae, systematics

## Introduction

As a part of a long-term project on insect-seed interactions, two of the authors (XYL, ZSX) have been investigating the diversity of insect seed predators of woody trees in a subtropical forest of Dujiangyan City (Sichuan Province, China) since 2002. The Dujiangyan region is in the northern part of the Hengduan Mountains, a biodiversity hotspot and priority area for biodiversity conservation in China. Located in the mountains on the western border of the Sichuan Basin, it is in an ecotone between two biogeographical regions, the Qinghai-Tibetan Plateau and the Chengdu Plain. Climatically, it lies in the middle subtropical zone, characterized by evergreen broad-leaved forests. After checking the weevil specimens obtained, a new species belonging to a new genus was identified. It was thought at first sight to belong to the tribe Rhynchitini (Attelabidae: Rhynchitinae). However, an in depth-study of the available material by another author (MAAZ) revealed that the new species belonged in fact to Deporaini. This weevil species was found to infest seedpods of *Callerya dielsiana* (Harms) P.K.Lôc ex Z.Wei & Pedley (Fabaceae) (Figs 1–2). In this study, a detailed description of this new genus and new species is provided, with supporting photographic material.

## Material and methods

On 27 October 2013, 262 weevil larvae were collected from seedpods of the plant *Callerya dielsiana* near Dujiangyan City (Sichuan province). All larvae were placed for adult emergence in a PVC tube (diameter 11 cm, length 40 cm) filled with local soil (30 cm in depth). We checked the emergence of adults once every week from March 2014, and collected adult specimens every day after the emergence of the first adult. In total, nine adult specimens emerged during June, July and September 2014. They were stored in 96% ethanol, and later seven were mounted for the morphological study, leaving two for molecular analysis.

The dry specimens show different degrees of immaturity, mainly affecting their abdomens. Only the male preserved in ethanol was mature enough to allow the extraction of moderately sclerotized genitalia and terminalia. This extraction was done directly in the conserving medium. The abdomen was then soaked overnight in lukewarm 10% sodium hydroxide for digestion of soft tissues. Genitalia and terminalia were photographed in glycerine and later mounted in DMHF (5,5-dimethyl-hydantoin formaldehyde resin) on an acetate card, and pinned together with the tergites and sternites. These have been cross labelled with the specimen in ethanol from which they were extracted.

Descriptions were made using a binocular Nikon SMZ 1500. Photographs (Figures 3–6) were taken with a Canon EOS 700D connected to a Canon MPE-65 lens, Figures 7–8 with a camera attached to a Leica M205 A stereoscopic microscope, Figures 9–10 with a Keyence VHX-1000C Large depth-of-field 3D Digital Microscope, Figures 11–16 with a Canon EOS 5D Mark II mounted on a Nikon SMZ 1500 stereoscopic



**Figures 1–2.** *Callerya dielsiana* **1** Bunch of pods in type locality of *Evemphyron sinense* **2** Opened ripe pod showing seed damage. Red arrows point to two larvae of *Evemphyron sinense*.

microscope, Figures 17–19 with an environmental scanning electron microscope FEI Inspect. Extended focus images were generated with Combine ZP 7.0 by Alan Hadley and edited with Adobe Photoshop CS 6.0 if required.

Original label data have been written below in Chinese script. Added transliterations into pinyin or translations are placed between square brackets. Data from different labels are separated by two slashes (//) and lines within a label by one slash (/).

Nomenclature follows Alonso-Zarazaga (2011) and in some cases Riedel (2014).

## Taxonomic treatment

### *Evemphyron* Alonso-Zarazaga, Lv & Wang, gen. n.

<http://zoobank.org/747DBE94-0E6E-4E94-8A24-D0C927515082>

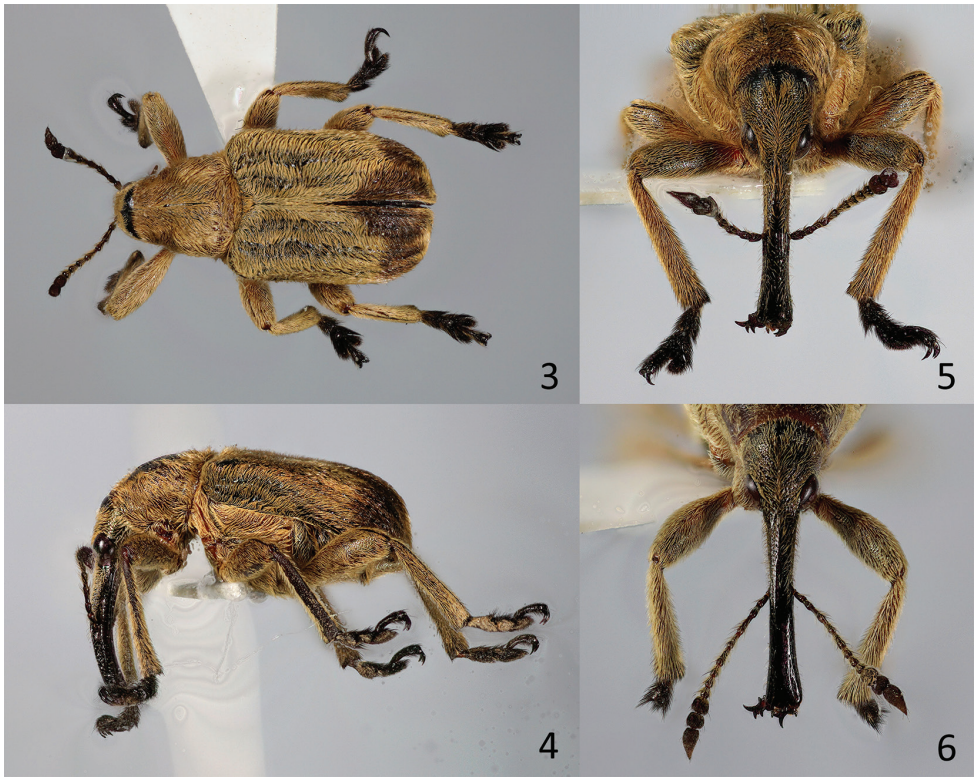
Figs 3–19

**Type species.** *Evemphyron sinense* Alonso-Zarazaga, Lv & Wang, sp. n.

**Description.** A member of the tribe Deporaini Voss, 1929 as currently understood (cf. Sawada 1993).

*Integument* black to brownish, with green to dark bronze metallic shine, some areas on legs, antennae and underside a little lighter.

*Vestiture* yellow or brown, dense; scales arched to oblique, piliform, those on elytra with apex sometimes flagelliform; brown scales forming a chevron on declivity; scales on dorsum of elytra placed transversally or pointing to outer apical angle of elytra, clearly subparallel to striae only on apical half of 1st interstria, those on anterior half of pronotum, head and metarostrum suberect and pointing forward; scape and funicle with yellow piliform scales, black setae at most as long as scales; tarsomeres densely covered with black piliform scales; very short suberect, arched black setae visible only

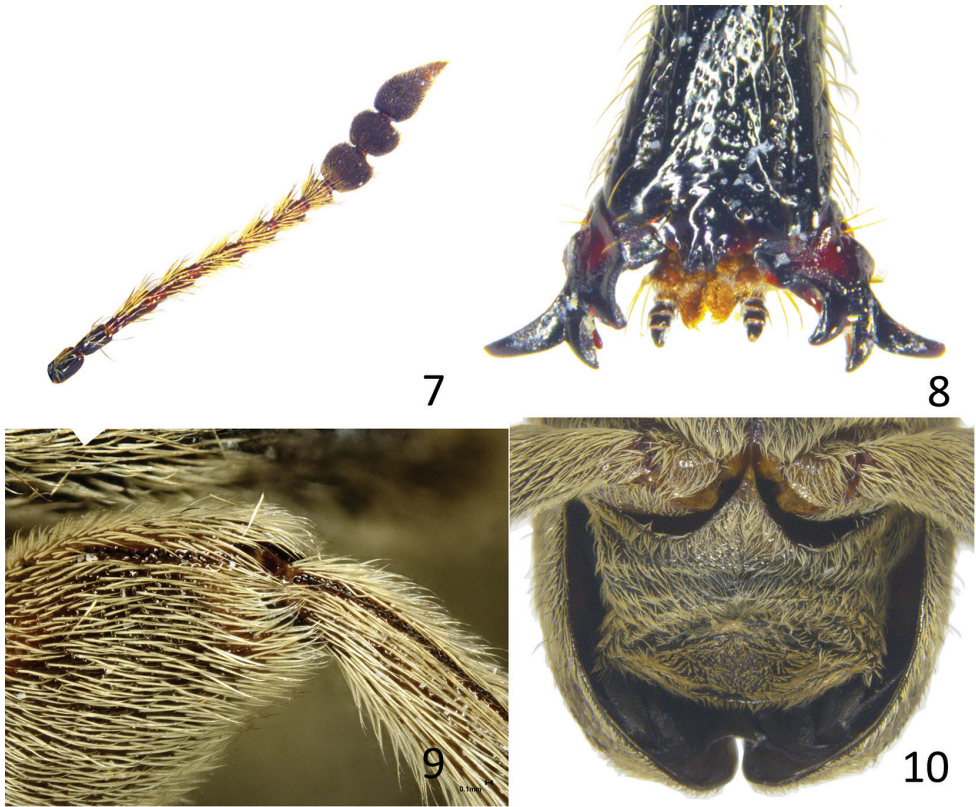


**Figures 3–6.** *Evemphyron sinense* **3** Male habitus, paratype, dorsal view **4** Male habitus, paratype, lateral view **5** Male rostrum, paratype, dorsal view **6** Female rostrum, paratype, dorsal view.

on apical two thirds of elytral interstriae 9 and 10, rarely visible on other interstriae, and on underside of rostrum.

**Mouthparts.** (Figs 7, 17–18) Mandibles with two teeth on outer margin, a short basal one and a long, sickle-shaped, outwardly-pointed, subapical one, this tooth caducous (in both sexes), leaving at most an obtuse tooth-like remainder after being shed (usually only in females); apex of right mandible with a small ventral cusp (usually quickly worn and obliterated in the apparently more aged specimens, like the outer basal teeth). Maxillary palpi well developed, projecting beyond apex of setose galea, 4-segmented, segments 1–3 transverse, segment 4 subconical, little shorter than wide at base, apex rounded, with eight longitudinal, rod-shaped sensilla. Prementum about as long as wide, asetose, tightly enclosed between deeply crescent-shaped anterior margin of postmentum, with a semicircular base, apex subtruncate, irregular; ligula densely setose, occupying entire apex of prementum; postmental apices almost reaching apices of ligular setae; labial palpi minute, 3-segmented, not protruding from notch in anterolateral corners of prementum and not projecting beyond its apex, first and second palpomeres each with one very long seta, third palpomere minute with two sensilla.





**Figures 7–10.** *Evemphyron sinense* **7** Apex of rostrum, paratype, dorsal view **8** Antenna **9** Metafemur and metatibia showing bracteate carina **10** Metacoxae and abdomen, male, ventral view.

*Rostrum* (Figs 5–6) elongate, in both sexes longer than pronotum, in side view with a strong lower lateral keel running more or less parallel to the ventral margin and a strong median ventral keel parallel to the other, leaving a sulcus between them. Lower margin of scrobe slightly prominent laterally at mesorostrum. Female metarostrum without dense patches of setae.

*Antennae* (Fig. 8) inserted a little behind middle in both sexes, more robust in male; scape shorter than mesorostral width; desmomeres circular in cross-section, 2<sup>nd</sup> desmome longer than either scape or first desmome, but shorter than length of both combined; club loose, slightly flattened, velvety, as long as last 4½ desmomeres, two first segments transverse, last segment longer than any of the others, but as long as or slightly shorter than first and second together, obpyriform, pointed, asymmetrical, its front margin straight to slightly concave, its hind margin convex.

*Head* moderately elongate, subglobose, very weakly constricted behind eyes in side view, but not in dorsal view. Eyes moderately convex, protruding from head outline, in dorsal view longer than minimum distance between them across forehead, in side view slightly oval.



*Pronotum* (Figs 3–4) rather isodiametric, widest in basal quarter, densely punctulate, with rounded, non-carinate sides and base curved towards scutellum, an incomplete median keel present, fine but marked. *Scutellum* (Figs 3–4) subrectangular, slightly transverse, densely punctulate and with dense vestiture.

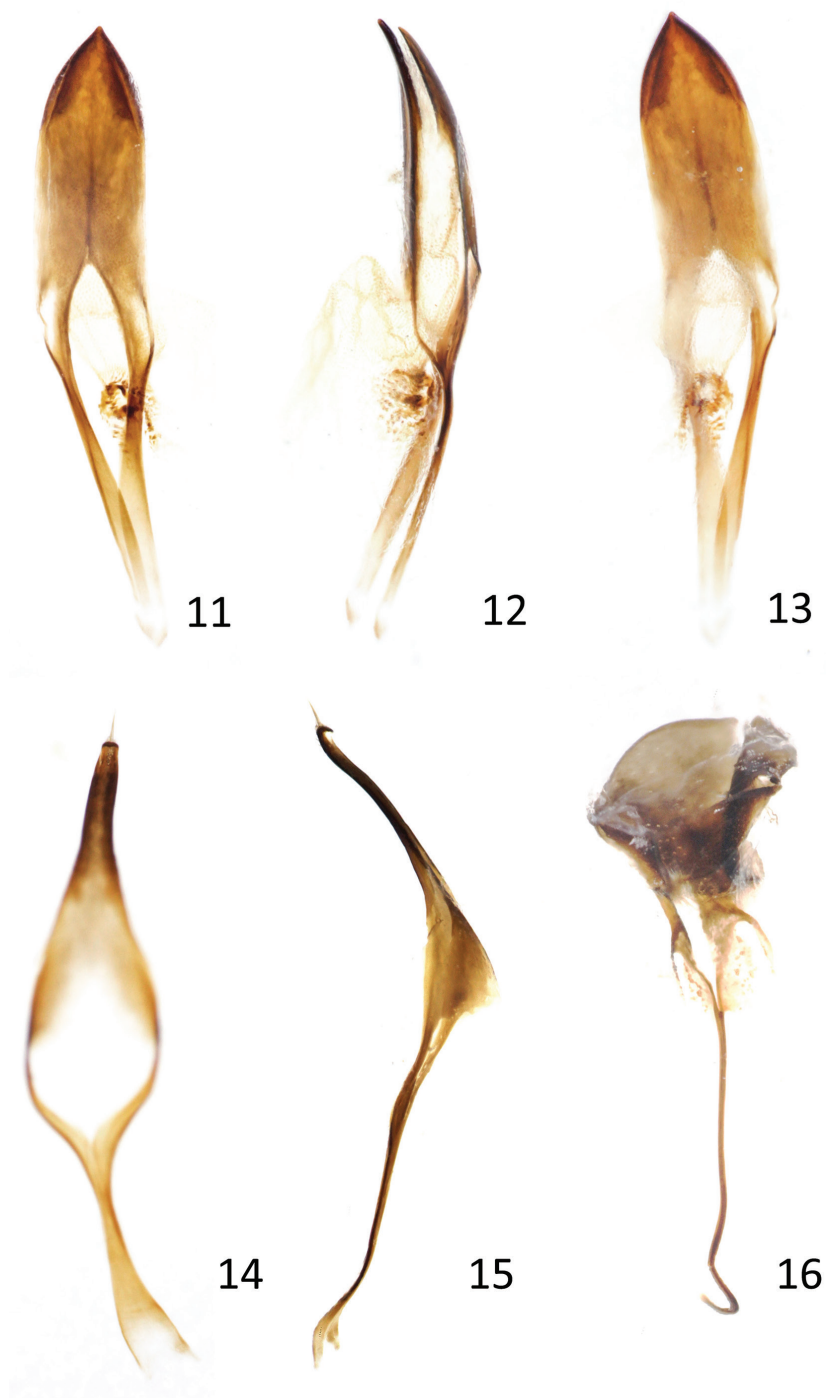
*Elytra* (Figs 3–4) oblong, dorsal surface flat to evidently concave behind scutellum, with rounded, developed humeri, bases obliquely converging towards scutellum, sides subparallel, falling almost vertically from 7th interstria to costal margin, elytral declivity very steep, elytral apices separately and widely rounded; ten striae formed by rows of strong, more or less rounded punctures, 9th and 10th striae confluent at metacoxal level; scutellar striole absent. Macropterous.

*Ventral areas.* Prosteronum short in both sexes, procoxae almost reaching front margin. Hypomera not touching at midline, sternellum large, separating both hypomera and forming part of prothoracic margin (Fig. 19). Procoxae projecting, subconical, tangential to one another. Male with rather large sex patch of setae on inner apex of procoxae. Mesocoxae separated by a distance of less than mesocoxal transverse diameter. Mesocoxal acetabuli open. Abdominal lobes absent, metacoxa reaching metanepisternum (Fig. 10). Tergites I–III fused. Sternites separated by a thin membrane, not visibly fused, sternite 1 barely longer than 2, sternite 5 as long as 4 in both sexes. Propygidium (tergite VI) almost completely covered by elytra, with sparse spicules not forming definite wing-folding patches. Pygidium (tergite VII) almost vertical, not costate, ca.  $1.25 \times$  as wide as long, in dorsal view covered by elytra in both sexes, but clearly visible from behind. Tergite VIII in male without bunches of macrosetae, these irregularly placed along margin.

*Legs.* Femora unarmed. Tibiae straight, moderately flattened, moderately widening towards apex, without mucrones or spurs in both sexes; meso- and metatibiae with an outer crenulate (bracteate) keel (Holloway 1984), this also present but reduced (bracteae scattered) on apical dorsum of meso- and metafemora, represented by a glabrous line (Fig. 9); protibiae with outer margin rounded. First tarsomere subtriangular, little longer than wide in all legs. Claws elongate, inner tooth three quarters as long as outer.

*Male genitalia and terminalia.* Penis (Figs 11–13) flattened, in dorsal view pedon apically pointed and mucronulate, tectum almost as wide as pedon, in side view ventral margin of pedon almost straight, weakly incurved at apex, temones moderately widening cephalad. Endophallus wider than tube of penis between the temones, endophallic armature consisting of sparse small denticles, these larger and condensed in an irregularly U-shaped patch between the base of the temones, around the gonopore, with a larger median projection near its anterior margin. Tegmen (Figs 14–15) with dorsal plate strongly projecting and tapering towards apex, this shortly recurved, with a few long and short macrochaetae, manubrium slightly asymmetrical, uniformly broadening to apex. Sternite IX fused to VIII, with apodeme strongly curving sinistro-anteriad near apex (Fig. 16).

*Female genitalia and terminalia.* Ovipositor with gonoxites very wide in anterior half, and obliquely narrowed to an elongate posterior half (“subdivided” in Sawada’s (1993) sense), styli cylindrical, ca.  $4 \times$  as long as wide. Spermatheca C-shaped, with



**Figures 11–16.** *Evemphyron sinense*, paratype male **11** Penis, dorsal view **12** Penis, lateral view **13** Penis, ventral view **14** Tegmen, dorsal view **15** Tegmen, lateral view **16** Sternite and tergite VIII (to the right) and sternite IX with apodeme turning sinistro-anterially at apex.

cornu robust, apically rounded, a little longer than body, no visible nodulus or ramus, ductus spermathecae and ductus glandulae very close to each other at junction with spermatheca. Sternite VIII with plate slightly longer than wide at base, rounded at apex, manubrium strongly curving sinistro-antieriad near apex.

**Etymology.** The genus name is based on the classical Greek prefix εὖ (well), latinized as *ev-* (as in *Evacanthus*) and the present active participle of the verb ἐμφύρω (to confuse), ἐμφύρων (the confusing one). Gender neuter. Stem is *Evemphyront-*.

**Chinese name.** 豆毛象属 [dòu máo xiàng shǔ].

***Evemphyron sinense* Alonso-Zarazaga, Lv & Wang, sp. n.**

<http://zoobank.org/57EF4590-5C0D-4B43-83F5-C2A7D4317ABD>

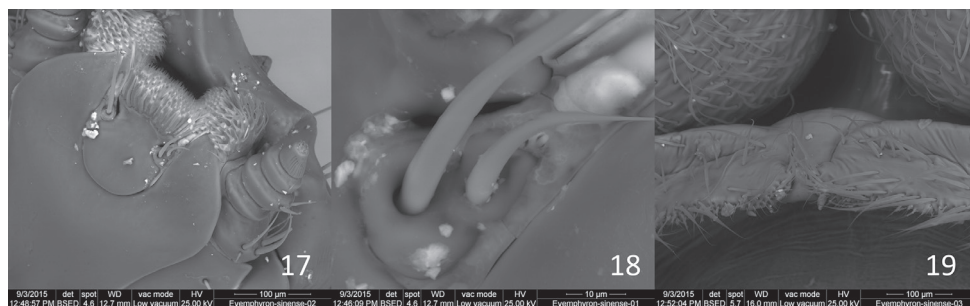
**Description.** Characters as given for the genus. In addition:

**Measurements** (in mm) (♂, n=5, ♀, n=2): Body length (standard, without head and rostrum): 6.76–7.33. Rostrum: length: 2.83–3.06 (♂), 3.43–3.46 (♀); width (apical): 0.60–0.67. Distance from antennal insertion to base: 1.13–1.33 (♂), 1.47 (♀). Forehead width: 0.53–0.67. Eye length: 0.67–0.73. Scape: 0.28 × 0.16 (♂), 0.28 × 0.12 (♀). Desmomerites: 1: 0.22 × 0.14 (♂), 0.24 × 0.13 (♀); 2: 0.32 × 0.14 (♂), 0.40 × 0.12 (♀); 3: 0.28 × 0.12 (♂), 0.34 × 0.12 (♀); 4: 0.34 × 0.14 (♂), 0.40 × 0.14 (♀); 5: 0.26 × 0.14 (♂), 0.24 × 0.14 (♀); 6: 0.24 × 0.14 (♂), 0.22 × 0.14 (♀); 7: 0.18 × 0.18 (♂), 0.20 × 0.16 (♀). Club: 1: 0.30 × 0.32 (♂), 0.30 × 0.30 (♀); 2: 0.28 × 0.34 (♂, ♀); 3: 0.54 × 0.30 (♂), 0.58 × 0.28 (♀). Pronotum: length: 2.33–2.43 (♂), 2.26–2.30 (♀); maximum width: 2.26–2.37. Elytra: length: 4.43–4.90; maximum width: 3.30–3.46.

**Rostrum** 1.17–1.27 × as long as pronotum in male (Fig. 5), 1.50–1.52 in female (Fig. 6), in dorsal view narrowest slightly behind antennal insertion, prorostrum widening towards apex, metarostrum towards base, apex rounded, medially with bidentate projection, prorostrum with one densely punctate lateral sulcus on each side and with dorsum densely punctulate in apical half, the punctures becoming sparser and larger behind, metarostrum with median, wide, impunctate and glabrous keel and two low lateral keels hidden under the dense, semierect, pointing forward scales; in side view, rostrum moderately curved, uniting with head at mid height of the latter, prorostrum tapering to apex; in ventral view with lateroventral keels marking the ventral borders of rostrum, and one low median keel having on each side a low sulcus with oblong punctures, these moderately setose.

**Antennae** inserted at 0.39–0.44 from base of rostrum in male, at 0.42–0.43 in female, articles with integument shining, with moderately long scales and setae, except velvety club, only with a few setae; scape and pedicel oblong, other desmomerites subcylindrical, except 7<sup>th</sup>, subglobular to suboblong.

**Head** with forehead forming a very obtuse to almost flat angle with rostrum in side view, forehead densely punctate and pubescent, scales pointing forward, underside of head with strong transverse rugae, these prominent in side view.



**Figures 17–19.** *Evemphyron sinense* **17** Apex of submentum, labium and maxilla **18** Detail of labial palpus **19** Detail of apex of hypomera and intervening sternellum.

*Pronotum* slightly depressed transversely behind front margin and before hind one, the punctures in the depressions more confused than in the remaining surface.

*Elytra* 1.33–1.43 × as long as wide, with interstriae densely punctulate, punctures ca. 1/6 the diameter of those forming the striae. Size of striae punctures decreasing towards apex of elytra.

*Legs* similar in both sexes, devoid of any sexual character.

*Wings* blackish.

**Material examined.** Nine specimens, rather teneral. All printed labels with 中国科学院动物研究所 [Zhōngguó kēxuéyuàn dòngwù yánjiū suǒ, Chinese Academy of Sciences, Institute of Zoology]. All specimens are deposited in this institution, except one male and one female paratype, which are deposited in the Alonso-Zarazaga collection (Museo Nacional de Ciencias Naturales, Madrid, Spain).

Holotype: 1 male, labelled: printed: 2014-VII-31 / 四川 都江堰 浦阳镇 花 / 溪村 [Sichuān, Dūjiāngyàn, Pǔyángzhèn, Huāxīcūn], 肖治术 [Xiāo Zhìshù] leg. // printed: N31°03'45.07" / E103°43'0.52" / Alt. 709 m // printed: 寄主 香花鸡血藤 [jìzhǔ [host] xiānghuā jī xuè téng] / *Callerya dielsiana* (Harms) // handwritten: A037 / 1(in red) ♂ / H.7.31.

Paratypes: 1 male, same data as holotype, except dated 2014-VII-12 and a handwritten label: A037, 14-7-12 / 山胡豆[Shānhú dòu], 左后[zuǒ hòu] / 足断[zú duàn] ♂; 1 male, same data as holotype, except dated 2014-VII-7 and a handwritten label: A037 / 1(in red) ♂ H.7.7 (specimen had been dissected, the abdomen had been discarded, some pieces in a glycerine vial); 1 male, same data as holotype, except dated 2014-VI-24, and a handwritten label: A037 / H / ♂ 6.24; 1 male, same data as holotype, except dated 2014-VII-7, and a handwritten label: A037 / 2 (in red) / ♂ H.7.7.; 1 female, same data as holotype, except dated 2014-VI-20, and a handwritten label: A037 / ♀ H.6.20; 1 female, same data as holotype, with a handwritten label: A037 / 2 (in red) / ♀ H.7.31 (specimen dissected, abdomen discarded, some pieces in a glycerine vial); 2 males, same data as holotype, but dated 2014-VI-12 and 2014-IX-17 respectively, conserved in pure ethanol vials for DNA extraction. (one of them, being less teneral, has been dissected for the study of the male genitalia and terminalia).

**Etymology.** The species is named after the country where it has been found, China (in Latin: *sinensis*, -e: Chinese). It is an adjective, in neuter form to agree with the gender of the genus.

**Chinese name.** 中华豆毛象 [zhōnghuá dòu máo xiàng].

**Host plant.** *Callerya dielsiana* (Harms) P.K.Lôc ex Z.Wei & Pedley (Fabaceae, Millettieae). Larvae develop in seeds inside the pods. This weevil species was not found on other plant species despite long-term collecting of several Fabaceae species and other possible host plants carried out at the same study site.

**Distribution.** This species is known only from the type locality in Sichuan Province (China).

## Discussion

This genus is superficially similar to *Cyllohynchites* Voss, 1930, mainly in the head not being constricted behind the eyes and the presence of dense yellowish piliform vestiture, somewhat reminiscent of that of *C. (C.) ursulus rostralis* (Voss, 1930), a common weevil in China. This led to the initial placement in the tribe “Rhynchitini”, *sensu* Riedel (2014). However, the characters of the minute labial palpi and the strongly crescentic apex of the postmentum are quite uncommon in that tribe, and more similar to the state found in members of the Deporaini (cf. Sawada 1987, 1988, 1993). Study of the male and female genitalia supported the latter placement, since the apodeme of male sternite IX and female sternite VIII curve sinistro-anteriad in both the new genus and the Deporaini. Thus Sawada’s (1988, 1993) characters defining the tribe Deporaini are partly met, even if the propygidium is hardly visible, the head is not constricted in dorsal view between vertex and occiput, and the labial palpi are 3-segmented.

The relationships of *Evemphyron* within Deporaini are also contentious. In fact, no genus known to belong to this tribe seems to be closely related. The keys provided by Legalov (2007) are confusing and do not help to locate a close genus, specimens of the new genus being taken to such disparate taxa as *Depasophilus* Voss, 1922, *Pseudocoenorrhinus* Voss, 1935, *Pseudodeporaus* Voss, 1922 or *Caenorhinus* s.l., depending upon which of the conflicting characters is given more strength.

The new genus should belong, according to the keys proposed by Legalov (2003, 2007) to the subtribe Depasophilina Legalov, 2003, defined by its author on a single character: the covered propygidium. The appreciation of this character is rather subjective, the placement of the latter depending on the maturity of the specimen and the way the specimen is prepared (pinned, glued, etc.). Legalov’s (2003) description of this subtribe can be applied to genera of the tribe Rhynchitini as well. No mention is made of the genitalic structures. Depasophilina are reddish, thinly pubescent, tropical animals of unknown habits. A close relationship of the new genus with them seems improbable.

If the character of the covered propygidium is not taken into consideration, the only possible placement is within the subtribe Deporaina. However, this is also a very disparate group regarding its contents. The combination of 3-segmented labial palpi,



**Table 1.** Character comparison between *Eusproda* and *Evemphyron* gen. n.

Character	<i>Eusproda</i>	<i>Evemphyron</i>
Integument	black with blue metallic shine mostly on elytra	black to brownish, with green to dark bronze metallic shine, some areas of appendages and abdomen lighter
Vestiture	sparse, thin, brown	dense, comprised of yellow and brown piliform scales
Base of rostrum in female	with dense long hairs	without long hairs
Labial palpi	2-segmented	3-segmented
Second desmomere	about as long as scape or 1 <sup>st</sup> desmomere	longer than scape or 1 <sup>st</sup> desmomere
Last antennal club segment	as long as 1 <sup>st</sup> or 2 <sup>nd</sup> , symmetrical	longer than 1 <sup>st</sup> or 2 <sup>nd</sup> , asymmetrical
Eye length in dorsal view	less than forehead width	more than forehead width
Pronotum	without median keel	with shortened median keel
Scutellum	oblong	slightly transverse
Elytra	elongate, ca. 1.7 × as long as wide	shorter, 1.33-1.43 × as long as wide
Elytra	uniformly convex	dorsally flat to concave behind scutellum
Elytral striae 9 <sup>th</sup> and 10 <sup>th</sup>	confluent near apex of elytra	confluent at metacoxal level
Propygidium	with wing folding patches	without wing folding patches
Metatarsomere 1	slightly longer than 2+3	clearly shorter than 2+3
Tegminal arms	broad, angulate	thin, curved
Tegminal manubrium	strongly asymmetrical, T-shaped at apex	slightly asymmetrical, uniformly broadened
Size	smaller (3.5-4.5 mm, without rostrum)	larger (6.76-7.33, without head and rostrum)
Biology	shoot-cutter	ovary- and young-fruit- driller

propygidium practically covered by the elytra and lacking definite wing folding patches, tibiae neither spurred nor mucronate in both sexes, long rostrum without long, dense patches of long hairs in female and male procoxae with sex patch does not indicate any included genus as an obvious close relative. This set of character states suggests that the new genus could be very primitive (or even the most primitive) in the subtribe, since the palpi segment number, the long rostrum and the male sex patch on the procoxae are clearly symplesiomorphies shared with genera of Byctiscini, Rhynchitini and Auletini, but they are absent from advanced members of Deporaini.

*Eusproda* shares with *Evemphyron* the male sex patch on the procoxae (a symplesiomorphy suggesting the basal position of both genera) and the trophic link to Fabaceae, although *Eusproda* behaves as a shoot cutter of kudzu (*Pueraria montana* (Lour.) Merr.) and Japanese clover (*Lespedeza cyrtobotrya* Miq.) (Fabaceae, Phaseoleae and Desmodieae, respectively), and not as an ovary or young fruit driller, as is the case for *E. sinense*. They also share the long rostrum, the very weak basal constriction of the head, the antennae inserted just behind middle of the rostrum, the non-contiguous hypomera, the subisodiametric pronotum, the absence of a scutellar striole, the tibiae without mucrones or spurs, overall similarity of the male genitalia and the ovipositor divided into two regions and with styli. Table 1 summarizes the differences between both genera.

*Evemphyron* could be close to any of the genera related to *Deporaus* Samouelle, 1819. However, the same combination of characters precludes the finding of another genus sharing putative synapomorphic features, as no known genus matches the combination found in *Evemphyron*. *Caenorhinus* C.G. Thomson, 1859 shares with *Evemphyron* the confluence of 9<sup>th</sup> and 10<sup>th</sup> elytral stria at metacoxal level and the absence of wing folding patches on the propygidium, but differs clearly by the presence of spurs in at least one pair of tibiae in both sexes and of mucrones at least in one pair of tibiae in males, the shorter, apically widened rostrum and the presence of defined endophallic sclerites.

In summary, this new genus is placed in Deporaini Deporaina on the basis of the minute labial palpi, the strongly crescentic apex of postmentum, the absence of scutellar striole, and the apodemes of male sternite IX and female sternite VIII curved sinistro-antieriad near their cephalic end.

The definitive placement of this new genus will have to wait until a molecular phylogeny of the tribe (and the subfamily Rhynchitinae as a whole) is performed.

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# Further contributions to the Hydradephaga (Coleoptera, Haliplidae, Gyrinidae and Dytiscidae) fauna of Prince Edward Island, Canada: new records, distributions and faunal composition

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## Abstract

The Haliplidae, Gyrinidae and Dytiscidae (Coleoptera) of Prince Edward Island, Canada were surveyed during the years 2004–2005. A total of 2450 individuals from 79 species were collected from 98 different localities, among which 30 species are newly recorded from that region. Among these, *Acilius sylvanus* Hilsenhoff, *Rhantus consimilis* Motschulsky and *Neoporus sulcipennis* (Fall) stand out as representing the easternmost reports of these species in Canada. Once removed, *Gyrinus aquiris* LeConte (Gyrinidae) is reinstated in the faunal list of Prince Edward Island. According to this study and literature 84 species of Hydradephaga are currently known from Prince Edward Island. The Nearctic component of the fauna is made up of 68 species (80.9%) and the Holarctic component of 16 species (19.1%). Most species are characteristic of the Boreal and Atlantic Maritime Ecozones and have a transcontinental distribution. In an examination of the Hydradephaga of insular portions of Atlantic Canada, we found that despite significantly different land areas and different distances to the neighbouring continental mainland the island faunas of Prince Edward Island and insular Newfoundland are very similar in the number of species (84 and 94 species respectively) despite differences in composition. With a land area significantly larger than that of Prince Edward Island, however, the fauna of Cape Breton Island was 39% smaller consisting of 53 species. This difference could be due to the comparative lack of collecting efforts on Cape Breton Island.

## Keywords

Coleoptera, Maritime Ecozone, Prince Edward Island, Hydradephaga, faunistic, biodiversity



## Introduction

The Maritime Provinces are a region of eastern Canada on the Atlantic coast consisting of New Brunswick, Nova Scotia, and Prince Edward Island. These provinces lie within the Atlantic Maritime Ecozone along with Québec's Gaspé Peninsula, Magdalen Archipelago and portions of the south shore of the St. Lawrence River. The climate of this ecozone is strongly influenced by the Atlantic Ocean, which produces cooler summers (average 14 °C) and warmer winters (average -5 °C), with coastal areas having slightly warmer winters and cooler summers than inland. The Atlantic Ocean also provides moisture to the region, producing mean precipitation of 900 mm a year inland and 1500 mm a year on the coast. Geologically, this region is a mix of sedimentary and igneous bedrock (Alarie 2009).

Prince Edward Island is located at 46 degrees latitude, 63 degrees longitude in the Gulf of St. Lawrence, off the Atlantic Coast of the Canadian mainland. This crescent shaped Island is only 224 km long and between 6 km and 64 km wide and is separated from the mainland by the Northumberland Strait. Its total land mass is 5,656 sq. km. The island has many wetlands and rivers, most of which are quite small. Often wide and relatively shallow many of these rivers experience a tidal influence over much of their length. Extensive salt and freshwater wetlands are, therefore, associated with several of the larger rivers.

Aquatic Adephaga have many morphological adaptations to their aquatic environment, making them excellent subjects for ecological and biogeographic studies (Moreno et al. 1997). Additionally, these beetles are important indicators of spatial and temporal changes in the environment. This is why some authors have used them as bio-indicators of habitat quality in terms of nutrient enrichment or the presence of potential pollutants (e.g., Arnott et al. 2006; Sánchez-Fernández et al. 2006). These ecosystems, however, are highly vulnerable to threats related to intensive human influences (Della Bella et al. 2005), thereof the importance of conducting faunistic surveys to help at documenting the diversity of these beetles.

Investigations of the Dytiscidae, Haliplidae, and Gyrinidae of the Maritime Provinces have been sporadic and regionally variable. Recent papers (Majka 2008; Majka and Kenner 2009; Majka et al. 2009; Alarie 2009; Webster 2008; Webster and DeMerchant 2012; Webster et al. 2016) resulted in a better understanding of the Hydradeephaga fauna in some areas. Despite many additions made recently by Majka (2008) the faunal list of Hydradeephaga of Prince Edward Island is deemed incomplete (Majka 2008). This study aims to fill this gap by presenting for the first time the results of an extensive field oriented research on the Hydradeephaga biodiversity of Prince Edward Island. It is conceivable that this study, complementing the previous works, should help to get a clearer picture of the diversity of Hydradeephaga on this region.

## Methods and conventions

### Study areas

Geologically, Prince Edward Island is part of the 'Maritimes Basin', a geographically low area that was filled hundreds of millions of years ago by sandy sediments eroded from the newly formed Appalachian Mountains to the south and west. Prince Edward Island's landscape has been largely influenced by the shape of the bedrock and by the ease with which it has been eroded. Low cliffs predominate along much of the shoreline especially on the northern headlands. The southern coastline, however, is more protected and erosion is, therefore, less pronounced. The several glaciers, which once covered Prince Edward Island, resulted in a nearly level to gently rolling landscape over much of the province. Approximately three-quarters of the land area is less than 50 meters above sea level, but a few hills throughout the central section of the Island have elevations of 150 meters (MacAlpine and Smith 2010).

Geographically, Prince Edward Island is subdivided into three counties: Kings, Prince, and Queens. Kings County is the province's smallest, most rural and least-populated county. That region is also least dependent upon the agriculture industry compared with the other two counties, while being more heavily dependent on the fishery and forest industry. Prince County is located in western part of the island; its defining geographic feature is Malpeque Bay, a sub-basin of the Gulf of St. Lawrence, which creates the narrowest portion of Prince Edwards's landmass. Much of Prince Edward Island's industrial base is concentrated in the eastern part of that county. Lastly, Queens County, located in central Prince Edward Island. The county is geographically divided by the Hillsborough River's estuary, a tidal inlet, which almost splits the county and Prince Edward Island. It is the richest and most populous county in the province. Queens County's geography varies from picturesque shorelines of sandstone cliffs, sandy beaches and sheltered bays on the Gulf of St. Lawrence and Northumberland Strait, to extensive farming operations throughout interior regions. Topography ranges from relatively flat to rolling hills in the central hill lands known as the Bonshaw Hills.

### Collecting methods

Collections were conducted over three periods, May 15–19, 2004, September 19–24, 2004 and September 17–21, 2005, which essentially reflects a similar collecting effort in each county. Sampling was unstructured and qualitative with the goal of obtaining a strict inventory of *Hydradephaga* of Prince Edward Island. Beetles were collected using D-net sweeps in a variety of microhabitats including macrophyte beds, rocky shores, organic-rich sediments, and open water. Overall 98 samples were obtained, which are listed in Table 1, along with locality data and habitat information.

**Table 1.** Prince Edward Island (Canada) sampling localities and habitats (2004–2005); letter in sample code refers to the county (K = Kings; P = Prince; Q = Queens).

Sample	Locality	Habitat
01K	Kings Co. South of Dover. 17.ix.2005	River, flowing into Murray River; saline at level of Mt Pleasant; shoreline densely covered with <i>Lemna</i> sp.
02K	Kings Co. Hwy 325, 1 km North of Hwy 17. 17.ix.2005	Pools in <i>Picea</i> sp. forest; densely covered with vegetation
03K	Kings Co. Jct Hwy 325 & Hwy 202. 17.ix.2005	Creek
04K	Kings Co. Hwy 325 2 km N. of jct to Hwy 202. 17.ix.2005	Shallow pond on sandy bottom
05K	Kings Co. Hwy 316 off Hwy 17. 18.ix.2005	Roadside fen with abundance of <i>Equisetum</i> sp.; dark yellow water with heavy accumulation of organic matter
06K	Kings Co. Hwy 318 off Hwy 17a. 18.ix.2005	<i>Sphagnum</i> sp. bog; sampling along shoreline under mats of shrubs
07K	Kings Co. S. of Hwy 324. 18.ix.2005	Eutrophic creek with <i>Typha</i> sp. and shrubs
08K	Kings Co. Hwy 17 S. of Hwy 324. 18.ix.2005	Creek with swift currents
09K	Kings Co. Hwy 17 S. of Hwy 324. 18.ix.2005	Woodland pool with heavy accumulation of organic matter, mainly dead leaves
10Q	Queen's Co. Off Hwy 4 near jct with Hwy 202. 18.ix.2005	<i>Sphagnum</i> sp. bog
11K	Kings Co. Hwy 2 near jct with Hwy 330. 19.ix.2005	Pond, shoreline with dense mats of Gramineae
12K	Kings Co. Chepstow at Hwy 16. 19.ix.2005	Small eutrophic creek in <i>Picea</i> sp. forest, slow moving water; samples from shallowest parts, under mats of dense vegetation
13K	Kings Co. Black Pond at Hwy 16, E. of Little Harbour. 19.ix.2005	Huge pond, located just beside sea
14K	Kings Co. MacVanes Creek at Hwy 16, east of Bothwell. 19.ix.2005	Eutrophic creek, almost still water
15K	Kings Co. Hwy 302 off Hwy 16. 19.ix.2005	Pond with <i>Nymphaea</i> sp.
16K	Kings Co. Hwy 302 off Hwy 16. 19.ix.2005	<i>Sphagnum</i> sp. bog; cold water; sampling under <i>Picea</i> sp. trees.
17K	Kings Co. Hwy 302 off Hwy 16. 19.ix.2005	Roadside ditch, at the edge of a bog; very dark brown water; presence of <i>Carex</i> sp. and <i>Typha</i> sp.
18K	Kings Co. Hwy 303 16 km W. of Hwy 16. 19.ix.2005	Fen; woodland pond with <i>Carex</i> sp. and Gramineae
19K	Kings Co. Hwy 303 12 km W. of Hwy 16. 19.ix.2005	Pond with <i>Nymphaea</i> sp.
20K	Kings Co. Hwy 306 3 km W. of Hwy 2. 20.ix.2005	Fen; densely covered with <i>Scirpus</i> sp.; moss along shoreline; slowly moving water; very dark brown water; <i>Acer rubrum</i> , <i>Picea</i> sp. and <i>Alnus</i> sp. forest
21K	Kings Co. Hwy 306 3 km W. of Hwy 2. 20.ix.2005	<i>Typha</i> sp. pond with abundance of <i>Equisetum</i> sp. in shallowest parts
22K	Kings Co. Hay River, W. of Clearspring at Hwy 6. 20.ix.2005	River with dark brown water; bed with large boulders
23K	Kings Co. Larkins Pond at Hwy 357, W. of Hwy 308. 20.ix.2005	Pond with clear water; shoreline with <i>Typha</i> sp.

Sample	Locality	Habitat
24K	Kings Co. Goose River Road, off Hwy 16. 20.ix.2005	Small pond with mats of <i>Carex</i> sp.
25K	Kings Co. Cable Head, Schooner Creek, at Hwy 16. 20.ix.2005	Eutrophic creek with swift current; beetles collected underneath banks
26K	Kings Co. Cable Head W. at Hwy 336. 20.ix.2005	Wetland with <i>Typha</i> sp. along shoreline
27K	Kings Co. Cable Head W. at Hwy 336. 20.ix.2005	<i>Sphagnum</i> sp. bog; abundance of Ericaceae
28K	Kings Co. Schooner River at Hwy 336. 20.ix.2005	River with very dark water
29K	Kings Co. Hwy 337, 1 km off Hwy 313. 21.ix.2005	Wetlands; sampling in mats of <i>Calamagrostis</i> sp.
30K	Kings Co. Junction Hwy 313 & Hwy 321. 21.ix.2005	Wetland in <i>Picea</i> sp. forest; dense accumulation of <i>Calamagrostis</i> sp., <i>Juncus</i> sp. and <i>Typha</i> sp.
31K	Kings Co. Jct Hwy 321 close to Martinvale. 21.ix.2005	Wetlands
32K	Kings Co. Hwy 320 near jct Hwy 322. 21.ix.2005	<i>Carex</i> sp. pools in <i>Picea</i> sp. forest; bed with black sediments
33K	Kings Co. Cherry Hills at Hwy 351. 21.ix.2005	Eutrophic Creek
34P	Prince Co. Tignish at Hwy 153. 15.v.2004	Permanent pond on clay bottom; at edge of <i>Picea</i> sp. and <i>Populus</i> sp. forest
35P	Prince Co. Donohue Rd, off Hwy 12, near Fisherman's Haven Provincial Park. 15.v.2004	Roadside ditch
36P	Prince Co. Green Mount at Hwy 162. 15.v.2004	<i>Typha</i> sp. pond
37P	Prince Co. Birch Groove Rd, off Hwy 153. 15.v.2004	Ephemeral woodland pool; dense accumulation of dead <i>Acer</i> sp. leaves; dark brown water
38P	Prince Co. Alberton, jct Hwy 150 and Hwy 12. 15.v.2004	Creek; heavily covered with <i>Carex</i> sp.
39P	Prince Co. Hwy 2, 1 km W. jct Hwy 151. 15.v.2004	Creek on sandy bed; sparse Gramineae along shoreline; very cold water
40P	Prince Co. Palmer Rd. 15.v.2004	Roadside woodland ditch, shallow, with heavy accumulation of organic debris; abundance of mosquito larvae
41P	Prince Co. Hwy 151, S. of Loretta. 16.v.2004	Creek, rocky bed, lacking vegetation
42P	Prince Co. Miminegash River at Hwy 151, near St. Lawrence. 16.v.2004	River
43P	Prince Co. Old Town Rd off Hwy 151. 16.v.2004	Roadside ditch; heavy accumulation of organic debris
44P	Prince Co. Old Town Rd off Hwy 151. 16.v.2004	Eutrophic creek; slow moving, dark brown water; heavy accumulation of organic debris
45P	Prince Co. Mill River at Hwy 148, near Howlan. 16.v.2004	River
46P	Prince Co. O'Leary, off Hwy 148. 16.v.2004	Shallow eutrophic creek
47P	Prince Co. Dublan, at Hwy 14. 16.v.2004	Pond; heavy accumulation of organic debris; dark brown water
48P	Prince Co. Hwy 142, near Roxberry. 17.v.2004	Permanent pond; heavy accumulation of <i>Sphagnum</i> sp., <i>Typha</i> sp., and <i>Scirpus</i> sp. along shoreline
49P	Prince Co. Hwy 137, off Hwy 142. 17.v.2004	Permanent <i>Sphagnum</i> sp. bog with <i>Ledum</i> sp., <i>Larix</i> sp., and <i>Carex</i> sp.; dark brown water

Sample	Locality	Habitat
50P	Prince Co. Hwy 138, 1 km off Hwy 2. 17.v.2004	Eutrophic ditch; heavy accumulation of organic debris; dark brown water
51P	Prince Co. Portage at Hwy 12. 17.v.2004	<i>Sphagnum</i> sp. bog lake
52P	Prince Co. Troy Rd. off Hwy 12. 17.v.2004	Shallow pond with mats of sedges and <i>Scirpus</i> sp.
53P	Prince Co. Hwy 2, 2 km W. jct Hwy 12. 18.v.2004	Permanent pond
54P	Prince Co. Hwy 12, near jct Hwy 131. 18.v.2004	Shallow creek, about 1 m wide; sandy bed
55P	Prince Co. Hwy 127, off Hwy 2, near St. Hubert 18.v.2004	Shallow creek, about 1 m wide; in <i>Abies balsamea</i> and <i>Betula alleghaniensis</i> forest; dense mats of bryophytes
56P	Prince Co. Jct Hwy 124 and Hwy 129. 18.v.2004	Road side ditch in <i>Betula papyrifera</i> , <i>Abies balsamea</i> and <i>Populus tremuloides</i> forest; heavy accumulation of organic debris; brown water
57P	Prince Co. Jct Hwy 124 & Hwy 129. 18.v.2004	Shallow creek, about 15 cm deep
58P	Prince Co. Evangeline, off Hwy 11. 18.v.2004	Shallow ephemeral ditch
59P	Prince Co. Hwy 165, 1km off Hwy 11. 18.v.2004	Roadside ditch; abundance of <i>Equisetum</i> sp. and Gramineae in <i>Acer</i> sp., <i>Abies balsamea</i> and <i>Betula papyrifera</i> forest
60P	Prince Co. St. Nicholas, off Hwy 11. 19.v.2004	<i>Sphagnum</i> bog in <i>Picea</i> sp. forest; dense accumulation of <i>Carex</i> sp. and bryophytes; dark brown water
61P	Prince Co. St. Nicholas, 1 km N. Hwy 11. 19.v.2004	<i>Sphagnum</i> bog lake in <i>Abies balsamifera</i> forest
62P	Prince Co. Hwy 122 off Hwy 2. 19.v.2004	Permanent pond; shallowest sections of pond with mats of Gramineae and sparse <i>Typha</i> sp.
63P	Prince Co. Hwy 123 off Hwy 12. 19.v.2004	Eutrophic creek with mats of Gramineae; slow moving water
64P	Prince Co. North of Miscouche, off Hwy 12. 19.v.2004	Large <i>Typha</i> sp. pond
65P	Prince Co. Hwy 122. 19.v.2004.	Shallow eutrophic roadside ditch
66Q	Queens Co. Irishtown at Hwy 104. 19.ix.2004	Creek, rocky bed; swift moving water
67Q	Queens Co. Henry Craig ln, off Branders Pond Rd. 1 km off Hwy 20. 19.ix.2004	Shallow creek on sandy beach with sparse <i>Typha</i> sp.
68P	Prince Co. Bedeque, Dunk River at Hwy 171. 19.ix.2004	River, on clay bed
69P	Prince Co. Searletown, jct Hwy 111 and Hwy 112. 20.ix.2004	Shallow creek on clay bed; slow moving water; dense vegetation
70P	Prince Co. Augustine Cove, jct Hwy 10 and Hwy 117. 20.ix.2004	Salted marsh
71P	Prince Co. North Tyron Memorial Park, off Hwy 115 N. 20.ix.2004	Eutrophic creek; boulders covered with algae
72P	Prince Co. Hwy 16 near South Melville. 20.ix.2004	Roadside ditch in <i>Picea</i> sp. forest
73P	Prince Co. Hwy 16 near South Melville. 20.ix.2004	Shallow creek on rocky bed; swift flowing, cold and clear water
74P	Prince Co. Hwy 101, 2 km off Hwy 2. 21.ix.2004	Creek; swift flowing water; discharge of pond; dense accumulation of vegetation
75Q	Queens Co. Park Corner, Shining Lake at Hwy 20. 21.ix.2004	Lake shoreline with <i>Typha</i> sp.



Sample	Locality	Habitat
76Q	Queens Co. Hwy 8, 2 km off Hwy 6. 21.ix.2004	Shallow pool with dense vegetation along shoreline
77P	Prince Co. Memorial Trail, Dunk River, at Hwy 109. 21.ix.2004	Small stream
78P	Prince Co. Wilms River, at Hwy 109. 21.ix.2004	Small stream
79Q	Queens Co. Hwy 6 near jct Hwy 233. 21.ix.2004	Creek
80Q	Queens Co. Rollings Pond near North Rustico. 21.ix.2004	Deep <i>Typha</i> sp. pond
81Q	Queens Co. North Rustico. 21.ix.2004	Shallow pond covered with vegetation
82Q	Queens Co. Hwy 225 off Hwy 114. 22.ix.2004	Pond; muddy bed
83Q	Queens Co. Breadalbane at Hwy 231. 22.ix.2004	Deep <i>Typha</i> sp. pond
84Q	Queens Co. Hunter River, Hwy 13, near Hwy 251. 22.ix.2004	Eutrophic creek, muddy bed, with algae
85Q	Queens Co. Hwy 15, near Tracadie. 22.ix.2004	Shallow depression covered with vegetation
86Q	Queens Co. Hwy 15, near Tracadie. 22.ix.2004	Pond besides sand dunes; shoreline with <i>Scirpus</i> sp. and <i>Typha</i> sp.
87Q	Queens Co. Hwy 15, near Tracadie. 22.ix.2004	Ericaceae and <i>Carex</i> sp. plain
88Q	Queens Co. Hwy 222, near Pleasant Groove. 22.ix.2004	Roadside ditch with <i>Scirpus</i> sp. and Gramineae
89Q	Queens Co. Canoe Cove at Hwy 19. 23.ix.2004	Shallow creek covered with vegetation
90Q	Queens Co. Hwy 19, 2 km W. Cumberland. 23.ix.2004	Shallow creek on rocky bed; mats of Gramineae; swift current
91Q	Queens Co. Bonshaw, off Hwy 1. 23.ix.2004	Pond
92Q	Queens Co. Green Bay Rd., off Hwy 237. 23.ix.2004	Small stream
93Q	Queens Co. Winsloe South, jct Hwy 223 and Hwy 256. 23.ix.2004	Shallow creek on rocky bed with dense vegetation
94Q	Queens Co. Winter River at Hwy 222. 23.ix.2004	River; slow moving water; dense vegetation along shoreline
95Q	Queens Co. Hwy 19 near Tracadie. 23.ix.2004	Creek; cold water with dense vegetation
96Q	Queens Co. Jct Hwy 271 and Hwy 218. 24.ix.2004	Marsh with <i>Alnus rugosa</i> and <i>Equisetum</i> sp. in <i>Picea</i> sp. forest
97Q	Queens Co. French Village, jct Hwy 217 and Hwy 218. 24.ix.2004	Marsh with <i>Scirpus</i> sp. and Ericaceae; dark brown water
98K	Kings Co. Hwy 320 off Hwy 22. 24.ix.2004	Small stream on rocky bed; abundance of <i>Alnus rugosa</i> along shoreline

## Nomenclature

Nomenclature is based on the classification in Oygur and Wolfe (1991) (Gyrinidae: *Gyrinus* Müller), Vondel (2005) (Halipilidae), Nilsson (2015) (Dytiscidae) and Gustafson and Miller (2015) (Gyrinidae: *Dineutus* MacLeay).

## Depositories

Voucher specimens are deposited in the author's research collection (Department of Biology, Laurentian University, Sudbury, Ontario).

Results

A total of 2450 specimens representing 79 species of Hydradephaga (9 Gyrinidae; 6 Haliplidae; 64 Dytiscidae) were collected in this study (Table 2). Among these, 30 species are reported for the first time and an additional one is reinstated in the faunal list of Prince Edward Island.

**Table 2.** Species of Hydradephaga (Dytiscidae, Gyrinidae, Haliplidae) collected in Prince Edward Island, Canada in 2004 and 2005 with sample numbers (as in Table 1), absolute (AF) and relative frequencies (%), and relative frequency of occurrence (RFO). Species and counties in bold denote new records from Prince Edward Island given in the present account.

Taxon	Sample numbers	AF (%)	RFO
<b>Gyrinidae</b>			
<i>Dineutus nigrrior</i> Roberts	19K, 47P, 48P, 49P, 61P, 62P	32 (1.31)	0.06
<i>Gyrinus affinis</i> Aubé	02K, 34P, 47P, 49P, 50P, 56P, 61P, 79Q, 94Q	21 (0.86)	0.09
<b><i>Gyrinus aquis</i> LeConte</b>	08K, 14K, 34P, 35P, 49P, 75Q, 78P, 87Q, 94Q, 95Q	147 (6.00)	0.10
<i>Gyrinus bifarius</i> Fall	28K	4 (0.16)	0.01
<i>Gyrinus confinis</i> Fall	13K, 33K, 75Q, 94Q	24 (0.98)	0.04
<b><i>Gyrinus latilimbus</i> Fall</b>	31K, 41P, 49P, 50P	5 (0.20)	0.04
<i>Gyrinus lecontei</i> (Hope)	02K, 28K, 34P, 50P, 56P, 61P, 62P, 63P, 65P, 79Q, 92Q, 98K	22 (0.90)	0.12
<b><i>Gyrinus pugionis</i> Fall</b>	31K, 38P, 47P, 49P	8 (0.33)	0.04
<i>Gyrinus sayi</i> Aubé	02K, 09K, 23K, 38P, 47P, 49P, 56P, 61P, 64P, 75Q, 79Q, 81Q, 87Q, 94Q, 95Q	61 (2.49)	0.15
<b>Haliplidae</b>			
<i>Haliphus canadensis</i> Wallis	48P	1 (0.04)	0.01
<b><i>Haliphus connexus</i> Matheson</b>	19K, 35P, 88Q	5 (0.20)	0.03
<i>Haliphus cribarius</i> LeConte	56P	1 (0.04)	0.01
<i>Haliphus immaculicollis</i> Harris	01K, 02K, 03K, 11K, 13K, 14K; 15K; 20K; 23K, 24K; 30K; 32K, 35P, 36P, 38P, 39P, 40P, 43P, 44P, 48P, 49P, 50P, 53P, 56P, 57P, 61P, 62P, 67Q, 71P, 73P, 76Q, 77P, 78P, 79Q, 81Q, 82Q, 83Q, 84Q, 87Q, 88Q, 91Q, 92Q, 93Q, 94Q, 98K	272 (11.10)	0.46
<i>Haliphus longulus</i> LeConte	35P, 37P, 49P, 65P, 02K, 05K, 06K, 17K	32 (0.13)	0.08
<i>Pelodytes edentulus</i> (LeConte)	39P, 45P, 56P, 67Q, 76Q	15 (0.61)	0.05
<i>Pelodytes tortulosus</i> Roberts	44P, 53P, 61P, 75Q, 02K	12 (0.49)	0.05
<b>Dytiscidae</b>			
<i>Acilius mediatius</i> (Say)	02K, 12K, 24K, 35P, 43P, 76Q	13 (0.53)	0.06
<i>Acilius semisulcatus</i> Aubé	09K, 15K, 24K, 27K, 32K, 34P, 43P, 50P, 52P, 62P, 78P, 81Q, 82Q, 88Q, 97Q	24 (0.98)	0.15
<b><i>Acilius sylvanus</i> Hilsenhoff</b>	34P	1 (0.04)	0.01
<i>Agabus ambiguus</i> (Say)	02K, 07K, 09K, 10Q, 12K, 13K, 17K, 18K, 19K, 27K, 32K, 35P, 36P, 38P, 46P, 55P, 62P, 67Q, 71P, 81Q, 82Q	60 (2.45)	0.21
<i>Agabus anthracinus</i> Mannerheim	01K, 02K, 05K, 06K, 07K, 09K, 10Q, 17K, 18K, 27K, 34P, 35P, 36P, 38P, 48P, 50P, 52P, 56P, 61P, 62P, 63P, 85Q, 87Q	87 (3.55)	0.24
<b><i>Agabus bifarius</i> (Kirby)</b>	06K, 27K, 32K, 52P	4 (0.16)	0.04
<b><i>Agabus erythropterus</i> (Say)</b>	02K, 11K, 12K, 31K, 32K	8 (0.33)	0.05

Taxon	Sample numbers	AF (%)	RFO
<i>Agabus phaeopterus</i> (Kirby)	38P	1 (0.04)	0.01
<i>Agabus punctulatus</i> Aubé	34P	9 (0.37)	0.09
<i>Agabus semipunctatus</i> (Kirby)	06K, 20K, 27K, 29K, 32K, 62P	9 (0.37)	0.06
<i>Agabus subfuscatus</i> Sharp	06K, 17K, 27K, 32K, 35P, 55P, 96Q	15 (0.61)	0.07
<i>Boreonectes griseostriatus</i> (DeGeer)	01K, 34P, 35P	10 (0.41)	0.03
<i>Colymbetes paykulli</i> Erichson	27K, 76Q	2 (0.08)	0.02
<i>Colymbetes sculptilis</i> Harris	09K, 18K, 20K, 21K, 26K, 27K, 34P, 73P, 78P	14 (0.57)	0.09
<i>Copelatus glypticus</i> (Say)	32K	2 (0.08)	0.01
<i>Coptotomus l. lenticus</i> LeConte	11K, 26K, 47P, 49P, 61P, 62P, 87Q, 97Q	12 (0.49)	0.08
<i>Desmopachria convexa</i> (Aubé)	02K, 05K, 27K, 43P, 44P, 48P, 52P, 60P, 97Q	27 (1.10)	0.09
<i>Dytiscus dauricus</i> Gebler	11K, 12K, 52P, 71P	4 (0.16)	0.04
<i>Dytiscus harisii</i> Kirby	51P	1 (0.04)	0.01
<i>Dytiscus verticalis</i> Say	17K, 18K, 49P, 56P, 62P, 84Q	6 (0.25)	0.06
<i>Graphoderus liberus</i> (Say)	49P, 61P	2 (0.08)	0.02
<i>Graphoderus perplexus</i> Sharp	61P, 97Q	3 (0.12)	0.02
<i>Hydaticus aruspex</i> Clark	09K, 27K, 35P, 37P, 60P	6 (0.25)	0.05
<i>Hydrocolus paugus</i> (Fall)	07K, 20K, 27K, 36P, 44P, 54P, 72P, 90Q, 98K	11 (0.45)	0.09
<i>Hydrocolus stagnalis</i> (Gemminger & Harold)	40P, 60P	2 (0.08)	0.02
<i>Hydroporus dentellus</i> Fall	02K, 04K, 05K, 06K, 15K, 27K	14 (0.57)	0.06
<i>Hydroporus fuscipennis</i> Schaum	21K, 23K, 27K, 32K, 34P, 35P, 36P, 40P	27 (1.10)	0.08
<i>Hydroporus gossei</i> Larson & Roughley	02K, 05K, 06K, 07K, 27K, 48P, 63P	21 (0.86)	0.07
<i>Hydroporus niger</i> Say	01K, 02K, 04K, 05K, 09K, 11K, 15K, 17K, 18K, 19K, 20K, 21K, 25K, 27K, 30K, 32K, 33K, 34P, 40P, 43P, 48P, 49P, 65P, 74P, 76Q, 88Q, 91Q, 97Q, 98K	97 (3.96)	0.30
<i>Hydroporus notabilis</i> LeConte	68P, 70P	2 (0.08)	0.02
<i>Hydroporus obscurus</i> Sturm	10Q, 11K	16 (0.65)	0.02
<i>Hydroporus signatus</i> Mannerheim	02K, 05K, 09K, 15K, 17K, 18K, 20K, 23K, 24K, 32K, 34P, 35P, 40P, 49P	37 (1.51)	0.14
<i>Hydroporus striola</i> (Gyllenhal)	02K, 05K, 06K, 09K, 11K, 12K, 13K, 15K, 18K, 20K, 21K, 27K, 29K, 30K, 32K, 37P, 40P, 48P, 49P, 52P, 58P, 59P, 60P, 72P, 81Q, 88Q	104 (4.25)	0.27
<i>Hydroporus tenebrosus</i> LeConte	01K, 04K, 13K, 18K, 20K, 21K, 27K, 28K, 35P, 36P, 40P, 52P, 58P, 81Q, 88Q	39 (1.59)	0.15
<i>Hydroporus tristis</i> (Paykull)	02K, 05K, 06K, 09K, 10Q, 15K, 21K, 25K, 27K, 32K, 35P, 40P, 44P, 60P	22 (0.90)	0.14
<i>Hygrotus compar</i> Fall	34P, 35P	6 (0.25)	0.02
<i>Hygrotus impressopunctatus</i> (Schaller)	09K, 34P, 35P, 36P, 60P, 68P, 76Q	29 (1.18)	0.07
<i>Hygrotus laccophilinus</i> (LeConte)	02K, 05K, 06K, 27K, 29K, 30K, 50P, 62P, 88Q	22 (0.90)	0.09

Taxon	Sample numbers	AF (%)	RFO
<i>Hygrotus picatus</i> (Kirby)	06K, 09K, 17K, 27K, 30K, 31K, 32K, 36P, 52P, 60P	27 (1.10)	0.10
<i>Hygrotus sayi</i> Balfour-Browne	02K, 05K, 09K, 11K, 13K, 14K, 15K, 21K, 23K, 26K, 27K, 36P, 37P, 44P, 48P, 49P, 50P, 51P, 52P, 53P, 56P, 61P, 62P, 67Q, 75Q, 76Q, 80Q, 81Q, 83Q, 88Q, 94Q	76 (3.10)	0.32
<i>Hygrotus turbidus</i> (LeConte)	09K, 17K, 18K, 27K, 35P, 50P, 56P, 62P	17 (0.69)	0.08
<i>Ilybiosoma seriatum</i> (Say)	08K, 12K, 16K, 38P, 39P, 41P, 44P, 46P, 54P, 55P, 69P, 71P, 73P, 76Q, 77P, 89Q, 98K	77 (3.14)	0.17
<i>Ilybius angustior</i> (Gyllenhal)	15K	1 (0.04)	0.01
<i>Ilybius biguttulus</i> (Germar)	01K, 02K, 04K, 05K, 12K, 13K, 15K, 17K, 19K, 23K, 24K, 27K, 30K, 31K, 44P, 48P, 50P, 56P, 68P, 75Q, 76Q, 81Q, 88Q, 91Q	67 (2.73)	0.25
<i>Ilybius discedens</i> Sharp	07K, 10Q, 20K, 27K, 51P, 88Q	7 (0.29)	0.06
<i>Ilybius erichsoni</i> Gemminger & Harold	34P, 37P, 57P	5 (0.20)	0.03
<i>Ilybius larsoni</i> (Fery & Nilsson)	07K, 10Q, 20K, 27K, 88Q	6 (0.25)	0.05
<i>Ilybius pleuriticus</i> (LeConte)	80Q, 83Q	2 (0.08)	0.02
<i>Laccophilus m. maculosus</i> Say	04K, 09K, 11K, 13K, 16K, 19K, 20K, 23K, 24K, 34P, 35P, 36P, 38P, 44P, 47P, 48P, 49P, 51P, 56P, 61P, 62P, 75Q, 76Q, 80Q, 81Q, 83Q, 88Q, 94Q, 97Q	69 (2.82)	0.30
<i>Laccornis latens</i> (Fall)	20K	2 (0.08)	0.01
<i>Liodesus affinis</i> (Say)	34P, 40P, 48P, 56P, 62P, 76Q, 78P, 88Q, 97Q	15 (0.61)	0.09
<i>Nebrioporus rotundatus</i> (LeConte)	01K, 14K, 22K, 28K, 29K, 38P, 42P, 45P, 64P, 66Q, 67Q, 71P, 74P, 76Q, 77P, 78P, 79Q, 83Q, 84Q, 92Q, 93Q, 94Q, 98K	162 (6.61)	0.24
<i>Neoporus carolinus</i> (Fall)	44P, 50P, 55P, 56P, 57P, 76Q	49 (2.00)	0.06
<i>Neoporus clypealis</i> (Sharp)	01K, 11K, 14K, 30K, 31K, 44P, 92Q, 93Q, 98K	22 (0.90)	0.09
<i>Neoporus dimidiatus</i> (Gemminger & Harold)	01K, 11K, 23K, 38P, 42P, 45P, 66Q, 71P, 74P, 77P, 78P, 79Q, 80Q, 81Q, 83Q, 93Q, 94Q, 98K	114 (4.65)	0.18
<i>Neoporus sulcipennis</i> (Fall)	42P, 45P	28 (1.14)	0.02
<i>Neoporus undulatus</i> (Say)	01K, 09K, 11K, 13K, 15K, 18K, 20K, 21K, 23K, 24K, 25K, 26K, 27K, 30K, 31K, 32K, 36P, 38P, 42P, 47P, 48P, 53P, 56P, 64P, 65P, 67Q, 75Q, 76Q, 78P, 80Q, 81Q, 83Q, 84Q, 86Q, 87Q, 88Q, 94Q	227 (9.27)	0.38
<i>Rhantus binotatus</i> (Harris)	09K, 15K, 20K, 24K, 26K, 35P, 36P, 44P, 48P, 49P, 62P, 72P, 76Q, 81Q, 83Q, 94Q	30 (1.23)	0.16
<i>Rhantus consimilis</i> Motschulsky	34P	5 (0.20)	0.01
<i>Rhantus sinuatus</i> (LeConte)	15K, 27K	5 (0.20)	0.02
<i>Rhantus suturellus</i> (Harris)	32K	1 (0.04)	0.01
<i>Rhantus wallisi</i> Hatch	53P, 61P	4 (0.16)	0.02
<i>Sanfillipodytes pseudovilis</i> (Young)	54P	1 (0.20)	0.01
	Total	2450	

## Faunistic and bionomics notes on the species newly collected from the Province of Prince Edward Island (Canada)

### GYRINIDAE

#### *Gyrinus aquiris* LeConte

**Note.** *Gyrinus aquiris* was the most abundant (45.4%) and one of the most frequently collected gyrinid (6%) in this survey. The species was collected in every county of Prince Edward Island (Table 2).

**Habitat.** In Prince Edward Island, *G. aquiris* was collected both from lotic (50%) and lentic (50%) environments (Tables 1–2), which is similar to the observations made by Alarie (2009) in the Magdalen Islands. Oygur and Wolfe (1991), however, reported 80% of specimens from lentic habitats whereas Morrisette (1979) reported the species from ponds and lakes invaded by aquatic plants.

**Distribution in the Maritime Ecozone.** *Gyrinus aquiris* was reported from the Magdalen Islands (Alarie 2009), and the Maritime Provinces of New Brunswick (Oygur and Wolfe 1991; Roughley 1991a; Webster 2016) and Nova Scotia (Campbell et al. 1987). Although Roughley (1991a) reported it from Prince Edward Island, Majka (2008) removed it from the province's faunal list since there were no voucher specimens or published records.

#### *Gyrinus latilimbus* Fall

**Note.** This very small gyrinid was collected in relatively low number (five specimens) at four different localities of Kings and Prince Counties (Table 2).

**Habitat.** Robert (1955) and Morrisette (1979) reported this species from small deep lakes near dense emergent vegetation (i.e., *Carex* and *Scirpus* spp.). Oygur and Wolfe (1991) reported it primarily (71%) from lentic habitats. The specimens collected in this survey were predominantly from lentic habitats (Tables 1–2).

**Distribution in the Maritime Ecozone.** *Gyrinus latilimbus* was reported from New Brunswick (Roughley 1991a; Webster 2016) and Nova Scotia (Roughley 1991a).

#### *Gyrinus pugionis* Fall

**Note.** Eight specimens were collected from four different localities of Kings and Prince Counties (Table 2).

**Habitat.** Robert (1955), Morrisette (1979) and Alarie (2009) reported *Gyrinus pugionis* from peaty or semi-peaty lakes; Robert (1955) mentioned that it invades narrow outflow streams from such lakes. Oygur and Wolfe (1991) reported it primarily (88%) from lentic habitats. In Prince Edward Island, most specimens were collected from lentic habitats (Tables 1–2).



**Distribution in the Maritime Ecozone.** This species was reported from the Provinces of New Brunswick (Roughley 1991a; Webster 2016) and Nova Scotia (Roughley 1991a), and from the Magdalen Islands (Alarie 2009).

## HALIPLIDAE

### *Haliplus connexus* Matheson

**Note.** Whereas found in relatively low numbers (5 specimens) *Haliplus connexus* is reported for in each of the three Prince Edward Island counties (Table 2).

**Habitat.** In Atlantic Canada, there are records from small streams, eutrophic ponds, river margins, slow streams and temporary habitats (Majka et al. 2009). In Prince Edward Island, all specimens of *Haliplus connexus* were collected along the edge of weedy lentic habitats (Tables 1–2).

**Distribution in the Maritime Ecozone.** The species is known also from the neighboring Maritime Provinces of New Brunswick and Nova Scotia (Matheson 1912; Wallis 1933; Roughley 1991b).

## DYTISCIDAE

### *Acilius sylvanus* Hilsenhoff

**Note.** This species is known from Prince Edward Island by a single specimen record from Prince County (Table 2).

**Habitat.** These beetles occur in the emergent zone of sun-warmed permanent or semi-permanent ponds in forested area (Larson et al. 2000). In Prince Edward Island, *A. sylvanus* was collected in a permanent pond on clay bottom located at the edge of a spruce and aspen forest (Tables 1).

**Distribution in the Maritime Ecozone.** This species is known only from the neighboring Province of New Brunswick (Webster 2008). The presence of *A. sylvanus* in Prince Edward Island represents the easternmost record of this species in Canada.

### *Agabus bifarius* (Kirby)

**Note.** This species is reported from four specimens collected in Kings (3) and Prince Counties (1) (Table 2).

**Habitat.** *Agabus bifarius* occurs amongst emergent grasses and sedges at the margin of a variety of lentic habitats, but it is especially characteristic of the margins of shallow, exposed, vernal ponds (Larson et al. 2000), which is essentially the type of habitats where it was found in Prince Edward Island (Table 1).

**Distribution in the Maritime Ecozone.** This species is also known from New Brunswick and Nova Scotia (Larson et al. 2000).

***Agabus erythropterus* (Say)**

**Note.** *Agabus erythropterus* is reported from eight specimens collected at five localities of Kings County (Table 2).

**Habitat.** This species is usually associated with slowly flowing water but occurs in depositional areas with thick organic silt (Larson et al. 2000), which is exactly the type of habitats where it was found in Prince Edward Island (Table 1).

**Distribution in the Maritime Ecozone.** *Agabus erythropterus* is also known from the neighboring Provinces of New Brunswick and Nova Scotia (Larson et al. 2000).

***Agabus punctulatus* Aubé**

**Note.** *Agabus punctulatus* is reported from nine specimens collected at one locality in Prince County (Table 2).

**Habitat.** The most typical habitat of this species is flooded grass along the margin of vernal ponds in open grassland areas (Larson et al. 2000), which is similar to the habitat where *A. punctulatus* was found in Prince Edward Island (Table 1).

**Distribution in the Maritime Ecozone.** This species is known also from the neighboring Maritime Provinces of New Brunswick and Nova Scotia (Larson et al. 2000).

***Agabus semipunctatus* (Kirby)**

**Note.** *Agabus semipunctatus* is reported from nine specimens from six localities of Kings and Prince Counties (Table 2).

**Habitat.** *Agabus semipunctatus* most often occurs in *Sphagnum* bogs but can also be collected regularly from *Carex*-choked waters (Larson et al. 2000). In Prince Edward Island, this species was essentially found in similar type of habitats (Table 1).

**Distribution in the Maritime Ecozone.** This species is also known from the neighboring Maritime Provinces of New Brunswick and Nova Scotia (Larson et al. 2000) and the Magdalen Islands (Alarie 2009).

***Colymbetes paykulli* Erichson**

**Note.** *Colymbetes paykulli* is reported from two specimens each collected in Queens and Kings Counties (Table 2).

**Habitat.** These beetles are almost entirely restricted to the cold water of *Sphagnum* bogs. They occur most frequently in small pools with emergent *Carex*, or along the edges of bog-ring lakes in embayments and beaver runs, often or near the willow zone (Larson et al. 2000). The two specimens collected in Prince Edward Island were from a *Sphagnum* bog and a shallow pool covered with vegetation respectively (Table 1).

**Distribution in the Maritime Ecozone.** This species is known also from the neighboring Maritime Provinces of New Brunswick and Nova Scotia (Larson et al. 2000).

### *Graphoderus liberus* (Say)

**Note.** *Graphoderus liberus* is reported from two specimens collected in Prince County (Table 2).

**Habitat.** These beetles generally occur in boggy (often brown water) ponds and lakes. They are usually found at the edge of the encircling bog in embayments or beaver runs (Larson et al. 2000). The two specimens collected in Prince Edward Island were from *Sphagnum* bog ponds (Table 1).

**Distribution in the Maritime Ecozone.** This species is known also from the neighboring Maritime Provinces of New Brunswick and Nova Scotia (Larson et al. 2000).

### *Hydrocolus paugus* (Fall)

**Note.** This species is reported from several specimens collected at various localities in each county of the province (Table 2).

**Habitat.** These beetles occur among moss or dense emergent vegetation and debris along the margins of small pools, ponds and springs. They occur in peatland as well as in sites where the water surface is shaded and the water is cool (Larson et al. 2000). Specimens were collected in Prince Edward Island from a variety of lentic and lotic habitats (Table 1).

**Distribution in the Maritime Ecozone.** This species is known also from the neighboring Maritime Provinces of New Brunswick and Nova Scotia (Larson et al. 2000), and the Magdalen Islands (Alarie 2009).

### *Hydroporus fuscipennis* Schaum

**Note.** This species is reported from several specimens collected at several localities of Prince and Kings Counties (Table 2).

**Habitat.** These beetles occur among dense emergent vegetation along the margins of small, often temporary pools, usually situated in grasslands, cleared areas or at the

edges of forest, but not typically in forest pools. *Hydroporus fuscipennis* is usually found in more or less eutrophic pools and is not common in peatlands (Larson et al. 2000). Specimens were collected in Prince Edward Island essentially from cattail ponds and roadside ditches (Table 1).

**Distribution in the Maritime Ecozone.** This species is known also from the neighboring Maritime Provinces of New Brunswick (Larson et al. 2000).

### *Hydroporus gossei* Larson & Roughley

**Note.** This species is reported from several specimens collected in Prince and Kings Counties (Table 2).

**Habitat.** In Newfoundland, this species has been collected from flooded grasses and emergent *Carex* along the margins of beaver ponds and roadside ponds. Specimens, which occurred on peaty substrates near boggy areas, were collected from areas exposed to the sun as well as from shorelines shaded by overhanging alder. In Prince Edward Island, *H. gossei* was collected in similar habitats, in addition to *Sphagnum* bogs and eutrophic creeks (Table 1).

**Distribution in the Maritime Ecozone.** This large, distinctive *Hydroporus* Clairville species has generally been confused with *H. rectus* Fall. In the Maritime ecozone, *H. gossei* is also reported from the neighboring Province of New Brunswick (Larson et al. 2000).

### *Hydroporus obscurus* Sturm

**Note.** This species is reported from 16 specimens collected at two localities of Prince and Kings Counties (Table 2).

**Habitat.** This species occurs in very small peatland pools, as well as in the moss mat along the margins of larger peatland pools and ponds (Larson et al. 2000). Specimens collected in Prince Edward Island were from a *Sphagnum* bog and the grassy shoreline of a pond (Table 1).

**Distribution in the Maritime Ecozone.** This species is known also from the neighboring Maritime Provinces of New Brunswick and Nova Scotia (Larson et al. 2000).

### *Hydroporus striola* (Gyllenhal)

**Note.** *Hydroporus striola* was one of the most abundant (4.25%) and most common species collected in the province (Table 2).

**Habitat.** *Hydroporus striola* occurs in almost all types of small, standing water where emergent vegetation is dense. Specimens occur in peatland pools, being most common in fen habitat and rather infrequent in ombrotrophic bog pools (Larson et

al. 2000). Specimens collected in Prince Edward Island were from an array of lentic habitats as described above (Table 1).

**Distribution in the Maritime Ecozone.** *Hydroporus striola* is the most ubiquitous species of *Hydroporus* in the boreal zone of the North Temperate Region (Larson et al. 2000). In the Maritime ecozone, this species is known also from the neighboring Provinces of New Brunswick and Nova Scotia (Larson et al. 2000), and the Magdalen Islands (Alarie 2009).

### *Hydroporus tenebrosus* LeConte

**Note.** Several specimens of *Hydroporus tenebrosus* were collected in each of the three counties of Prince Edward Island (Table 2).

**Habitat.** This species occurs mainly in forested regions but is also common in the prairie parkland. Beetles occur in the emergent zone of small, usually temporary pools and ponds. They are usually found in eutrophic, sun-warmed sites (Larson et al. 2000). In Prince Edward Island, *H. tenebrosus* was almost essentially collected only in ponds, ephemeral pools, and roadside ditches (Table 1).

**Distribution in the Maritime Ecozone.** The species is known also from the neighboring Provinces of New Brunswick and Nova Scotia (Larson et al. 2000), and the Magdalen Islands (Alarie 2009).

### *Hydroporus tristis* (Paykull)

**Note.** Several specimens of *Hydroporus tristis* were collected from Kings and Prince Counties (Table 2).

**Habitat.** This species occurs in a variety of lentic habitats. Specimens are usually found among dense emergent vegetation at the margin of small, often more or less shaded pools. *Hydroporus tristis* is also common in small peatland pools (Larson et al. 2000). Whereas collected in few eutrophic creeks in Prince Edward Island, *H. tristis* was most generally found in ponds and shallow pools characterized by heavy accumulation of organic debris as well as in *Sphagnum* bogs (Table 1).

**Distribution in the Maritime Ecozone.** The species is known also from the neighboring Province of New Brunswick (Larson et al. 2000), and the Magdalen Islands (Alarie 2009).

### *Hygrotus compar* Fall

**Note.** *Hygrotus compar* is reported from six specimens collected Prince County (Table 2).

**Habitat.** In the Prairies, *H. compar* has been recorded from the margin of temporary ponds, usually in fresh water but specimens have also been found in saline ponds



(Larson et al. 2000). In Prince Edward Island, specimens were found in a roadside ditch and a clay bed pond at the edge of a spruce and aspen forest (Table 1).

**Distribution in the Maritime Ecozone.** The species is known also from the neighboring Province of New Brunswick (Larson et al. 2000).

### *Hygrotus laccophilinus* (LeConte)

**Note.** *Hygrotus laccophilinus* is reported from 22 specimens collected throughout the province (Table 2).

**Habitat.** This species has been collected from a variety of small ponds. It appears to be most common among emergent grasses and sedges along the margin of permanent, or at least long lasting ponds. Specimens have been found most frequently in ponds in open country cleared of forest but they have also been collected in woodland ponds, especially beaver ponds (Larson et al. 2000). In Prince Edward Island, specimens were most frequently found in ponds and roadside ditches (Table 1).

**Distribution in the Maritime Ecozone.** The species is known also from the neighboring Provinces of New Brunswick and Nova Scotia (Larson et al. 2000).

### *Ilybius discedens* Sharp

**Note.** Although found in relatively low numbers (seven specimens), this species is reported from each of the three Prince Edward Island counties (Table 2).

**Habitat.** *Ilybius discedens* is one of the most characteristic water beetle species of boreal peatland generally occurring in small, moss-ringed pools, often where the water is cold to the touch (Larson et al. 2000). In Prince Edward Island, the majority of specimens were collected from *Sphagnum* bogs, although a few specimens were associated with eutrophic lotic habitats (Table 1).

**Distribution in the Maritime Ecozone.** This species is also known from the neighboring Provinces of New Brunswick and Nova Scotia (Larson et al. 2000), and the Magdalen Islands (Alarie 2009).

### *Ilybius erichsoni* (Gemminger & Harold)

**Note.** This species is reported from three localities of Prince County (Table 2).

**Habitat.** This is a species of forested regions where it occurs amongst dense vegetation, usually *Carex*, at the margins of both temporary and permanent ponds (Larson et al. 2000). Except for one specimen, which was collected in a shallow creek, all specimens of *Ilybius erichsoni* were collected in the type of habitats mentioned by Larson et al. (2000) (Table 1).

**Distribution in the Maritime Ecozone.** This species is known also from the neighboring Provinces of New Brunswick and Nova Scotia (Larson et al. 2000), and the Magdalen Islands (Alarie 2009).

### *Ilybius larsoni* (Fery & Nilsson)

**Note.** This species is reported from five localities of Queens and Kings Counties (Table 2).

**Habitat.** Specimens of *Ilybius larsoni* have generally been collected from small, cold woodland pools, usually with moss and accumulation of plant debris such as grass or sedge stalks or fallen leaves. Specimens have also been collected from small, peat-rich springs (Larson et al. 2000). In Prince Edward Island, this species was found in *Sphagnum* bogs and eutrophic creek (Table 1).

**Distribution in the Maritime Ecozone.** This species is known also from the neighboring Provinces of New Brunswick and Nova Scotia (Larson et al. 2000).

### *Laccornis latens* (Fall)

**Note.** *Laccornis latens* is reported from two specimens collected in Kings County (Table 2).

**Habitat.** The most common habitat for this species is semi-permanent, cool, shaded pools with deep leaf debris but few vascular plants; moss is often abundant (Larson et al. 2000). In Prince Edward Island, the two specimens sampled were collected within shoreline moss in a fen densely covered with *Scirpus* (Table 1).

**Distribution in the Maritime Ecozone.** This species is known also from the neighboring Provinces of New Brunswick and Nova Scotia (Larson et al. 2000).

### *Neoporus carolinus* (Fall)

**Note.** *Neoporus carolinus* is reported from several specimens collected in Prince and Queens Counties (Table 2).

**Habitat.** This species occurs among emergent vegetation such as sedges (Larson et al. 2000). In Prince Edward Island, beetles were collected from both lotic and lentic habitats, most of which characterized by dark brown water (Table 1).

**Distribution in the Maritime Ecozone.** *Neoporus carolinus* is known also from the neighboring Provinces of New Brunswick and Nova Scotia (Larson et al. 2000).

### *Neoporus clypealis* (Sharp)

**Note.** *Neoporus clypealis* is reported from 22 specimens collected in each of the three Prince Edward Island counties (Table 2).

**Habitat.** This species occurs among emergent vegetation such as sedges, along the margins of slow, marshy streams, beaver ponds, and small lakes. Beetles are generally found where there is some water movement and are usually on mineral substrates (Larson et al. 2000). With few exceptions, most specimens collected in Prince Edward Island were from creeks and small rivers (Table 1).

**Distribution in the Maritime Ecozone.** *Neoporus clypealis* is known also from the neighboring Provinces of New Brunswick and Nova Scotia (Larson et al. 2000).

### *Neoporus sulcipennis* (Fall)

**Note.** Several specimens of *Neoporus sulcipennis* were collected at two localities of Prince County (Table 2).

**Habitat.** This species occurs in small to medium sized warm, clear streams, often in depositional areas along the stream margins (Larson et al. 2000). In Prince Edward Island, all specimens were collected along the margins of rivers (Table 1).

**Distribution in the Maritime Ecozone.** *Neoporus sulcipennis* is known also from the neighboring Province of New Brunswick (Larson et al. 2000). The presence of this species in Prince Edward Island represents the easternmost record in Canada.

### *Rhantus consimilis* Motschulsky

**Note.** *Rhantus consimilis* is reported from five specimens collected at the same locality of Prince County (Table 2).

**Habitat.** This species occurs in warm, weedy ponds, generally in open grassland areas. It has been collected from both permanent and temporary habitats (Larson et al. 2000). The specimens collected in Prince Edward Island were from a permanent pond located at the edge of a spruce and willow forest (Table 1).

**Distribution in the Maritime Ecozone.** Prior to this study, *R. consimilis* had only been reported in New Brunswick (Webster 2008). The presence of this species in Prince Edward Island is its easternmost report in Canada.

### *Rhantus sinuatus* (LeConte)

**Note.** Five specimens of *R. sinuatus* were collected at two localities of Kings County (Table 2).

**Habitat.** This species occurs in lentic habitats densely overgrown with emergent vegetation. The species is common in bogs, but it also occurs in habitats with dense sedges, rushes or *Typha* (Larson et al. 2000). In Prince Edward Island, specimens were collected from a permanent pond with *Nymphaea* and a *Sphagnum* bog (Table 1).

**Distribution in the Maritime Ecozone.** *Rhantus sinuatus* is known also from the neighboring Provinces of New Brunswick and Nova Scotia (Larson et al. 2000).

***Rhantus suturellus* (Harris)**

**Note.** One specimen of *R. suturellus* was collected in Kings County (Table 2).

**Habitat.** These beetles occur in cold, densely shaded water in forested areas, frequently found in bogs and fens (Larson et al. 2000). In Prince Edward Island, the specimen collected was found in a *Carex* pool with black sediments in a spruce forest (Table 1).

**Distribution in the Maritime Ecozone.** *Rhantus suturellus* is known also from the neighboring Provinces of New Brunswick (Webster 2008) and Nova Scotia (Larson et al. 2000) and the Magdalen Islands (Alarie 2009).

***Sanfilippodytes pseudovilis* (Young)**

**Note.** One specimen of *S. pseudovilis* was collected in Prince County (Table 2).

**Habitat.** The species is very common in cold stenothermal springs where it can be taken either in the limnocene pools or among the mosses along the spring margin (Larson et al. 2000). In Prince Edward Island, the only specimen collected was found in a narrow and shallow creek flowing on a sandy bed (Table 1).

**Distribution in the Maritime Ecozone.** This species is also known from the neighboring Province of New Brunswick (Webster 2008) and the Magdalen Islands (Alarie 2009).

**Discussion**

A total of 79 Hydradephaga species was recovered from 98 samples during a survey conducted on Prince Edward Island, Canada, between 2004–2005. Included among these were 30 new provincial records consisting of one haliplid, 2 gyrids and 27 dytiscids (Table 2). *Acilius sylvanus*, *Rhantus consimilis* and *Neoporus sulcipennis* stand out as representing the easternmost reports of these species in Canada. In addition to these new records, one species, *Gyrinus aquiris* (Gyrinidae), which had been removed by Majka (2008), is reinstated in the faunal list of Prince Edward Island. According to this study and literature, 84 species of Hydradephaga are currently known from Prince Edward Island (Table 3). There are records of 67 species from Queens County, 66 from Kings County and 58 from Prince County.

The Nearctic component of the fauna of Prince Edward Island is made up of 68 species (80.9%), the Holarctic component of 16 species (19.1%). Most species are characteristic of both the Boreal and Atlantic Maritime Ecozones and have a trans-continental distribution, except for *Acilius medius* (Say), *A. sylvanus*, *Agabus erythropterus*, *A. subfuscatus* Sharp, *Copelatus glyphicus* (Say), *Dineutus nigrior* Roberts, *Dytiscus verticalis* Say, *Ilybius biguttulus* (Germar), *I. larsoni*, *Gyrinus lecontei* (Hope), *Haliplus connexus*, *Hydroporus gossei*, *H. niger* Say, *Laccophilus m. maculosus* Say, *Laccornis latens*,

**Table 3.** Checklist of species of Hydradephaga recorded from Prince Edward Island, Canada, and their provincial and territorial distribution within northeastern North America (NA). \*, Holarctic species; †, species not collected in this survey but recorded in Majka (2008); K, Kings County; P, Prince County; Q, Queens County.

Taxon	Counties			Distribution in northeastern NA
	P	Q	K	
<b>GYRINIDAE</b>				
<b>Gyrininae</b>				
Dineutini				
<i>Dineutus assimilis</i> Kirby†		1		CT, ME, NB, NS, ON, PE, QC, RI
<i>Dineutus hornii</i> Roberts†		1	1	CT, MA, ME, MI, NB, NH, NS, NY, ON, PE, QC, RI
<i>Dineutus nigrior</i> Roberts	1		1	CT, MA, ME, MI, NB, NH, NS, ON, PE, QC, RI
Gyrinini				
<i>Gyrinus affinis</i> Aubé	1	1	1	LB, MA, ME, NB, NE, NH, NS, NY, ON, PE, QC, RI, VT
<i>Gyrinus aquiris</i> LeConte	1	1	1	LB, MA, ME, MI, NB, NE, NS, NY, ON, PE, QC, RI
<i>Gyrinus bifarius</i> Fall	1	1	1	LB, MA, ME, NB, NE, NH, NS, NY, ON, PE, QC
<i>Gyrinus confinis</i> Fall		1	1	CT, LB, MA, ME, NB, NE, NH, NS, NY, ON, PE, QC, SM, VT
<i>Gyrinus fraternus</i> Couper†		1		MA, ME, NB, NH, NS, NY, ON, PE, QC, VT
<i>Gyrinus latilimbus</i> Fall	1		1	CT, LB, MA, ME, NB, NE, NH, NS, NY, ON, PE, QC, SE
<i>Gyrinus lecontei</i> (Hope)	1	1	1	CT, MA, ME, NB, NH, NS, NY, ON, PE, QC, RI
<i>Gyrinus pugionis</i> Fall	1		1	MA, ME, MI, NB, NH, NS, NY, ON, PE, QC, VT
<i>Gyrinus sayi</i> Aubé	1	1	1	CT, MI, LB, MA, ME, NB, NE, NH, NS, NY, ON, PE, QC, RI, SM
<b>HALIPLIDAE</b>				
<i>Haliphus canadensis</i> Wallis	1		1	MA, NB, NS, ON, PE, QC
<i>Haliphus connexus</i> Matheson	1	1	1	CT, MA, ME, NB, NH, NS, NY, ON, PE, QC, VT
<i>Haliphus cribarius</i> LeConte	1	1	1	CT, LB, MA, ME, MI, NB, NE, NH, NS, NY, ON, PE, QC, SM
<i>Haliphus immaculicollis</i> Harris	1	1	1	CT, LB, MA, ME, MI, NB, NE, NH, NS, NY, ON, QC, PE, RI, SM, VT
<i>Haliphus longulus</i> LeConte	1	1	1	MA, ME, NB, NH, NB, NS, NY, ON, PE, QC, RI
<i>Peltodytes edentulus</i> (LeConte)	1	1		MA, NB, NH, NS, ON, QC, PE, RI
<i>Peltodytes tortulosus</i> Roberts	1	1	1	ME, NB, NH, NS, NY, ON, PE, QC
<b>DYTISCIDAE</b>				
<b>Agabinae</b>				
<b>Agabini</b>				
<i>Agabus ambiguus</i> (Say)	1	1	1	LB, ME, MI, NB, NE, NH, NS, ON, PE, QC, RI, SM
<i>Agabus anthracinus</i> Mannerheim	1	1	1	LB, MA, ME, MI, NB, NE, NH, NS, NY, ON, PE, QC, SM, VT
<i>Agabus bifarius</i> (Kirby)*	1		1	LB, MA, ME, NB, NH, NS, NY, ON, PE, QC, RI
<i>Agabus discolor</i> (Harris)* †		1		LB, MA, ME, NB, NH, NS, NY, ON, PE, QC, VT
<i>Agabus erythropterus</i> (Say)			1	CT, LB, MA, ME, NB, NE, NS, NY, ON, PE, QC, RI
<i>Agabus phaeopterus</i> (Kirby)	1	1		LB, MA, ME, MI, NB, NE, NH, NS, NY, ON, PE, QC
<i>Agabus punctulatus</i> Aubé	1			LB, MA, ME, NB, NH, NS, ON, PE, QC, RI
<i>Agabus semipunctatus</i> (Kirby)	1		1	CT, LB, MA, ME, MI, NB, NE, NH, NS, NY, ON, QC, RI
<i>Agabus subfuscatus</i> Sharp	1	1	1	CT, LB, MA, ME, NB, NE, NH, NS, ON, PE, QC, VT
<i>Ilybiusoma seriatum</i> (Say)	1	1	1	CT, LB, MA, ME, MI, NB, NE, NH, NS, ON, PE, QC, SM



Taxon	Counties			Distribution in northeastern NA
	P	Q	K	
<i>Ilybius angustior</i> (Gyllenhal)*		1	1	LB, MI, ME, NB, NE, NH, NS, ON, PE, QC, SM, VT
<i>Ilybius biguttulus</i> (Germar)	1	1	1	MA, ME, MI, NB, NE, NH, NS, NY, ON, PE, QC, RI, SM, VT
<i>Ilybius discedens</i> Sharp	1	1	1	LB, ME, MI, NB, NE, NH, NS, ON, PE, QC, SM
<i>Ilybius erichsoni</i> Gemminger & Harold*	1			LB, MA, ME, MI, NB, NE, NH, NS, NY, ON, PE, QC
<i>Ilybius larsoni</i> (Fery & Nilsson)		1	1	NB, NS, ON, PE, QC
<i>Ilybius pleuriticus</i> (LeConte)		1		CT, LB, MA, ME, MI, NB, NE, NS, ON, PE, QC, RI, SM, VT
Colymbetinae				
Colymbetini				
<i>Colymbetes paykulli</i> Erichson*		1	1	LB, ME, NB, NE, NS, ON, PE, QC
<i>Colymbetes sculptilis</i> Harris	1	1	1	CT, LB, MI, NB, NE, NH, NS, NY, ON, PE, QC, RI
<i>Rhantus binotatus</i> (Harris)	1	1	1	CT, LB, ME, MI, NB, NE, NH, NS, ON, PE, QC, RI, SM
<i>Rhantus consimilis</i> Motschulsky	1			MA, ME, NB, NH, NY, PE, QC, VT
<i>Rhantus sinuatus</i> (LeConte)			1	LB, MA, ME, NB, NE, NH, NS, NY, ON, PE, QC
<i>Rhantus suturellus</i> (Harris)*			1	CT, LB, MA, ME, MI, NB, NE, NH, NS, NY, ON, PE, QC, SM
<i>Rhantus wallisi</i> Hatch	1	1		LB, MA, MI, NB, NE, NH, NS, ON, PE, QC, SM
Copelatinae				
Copelatini				
<i>Copelatus glyphicus</i> (Say)			1	CT, ME, NE, NH, NB, NS, ON, PE, QC, RI
Coptotominae				
Coptotomini				
<i>Coptotomus l. lenticus</i> LeConte	1	1	1	MA, ME, MI, NB, NH, NS, NY, ON, QC, NB, PE, RI
Dytiscinae				
Aciliini				
<i>Acilius mediatius</i> (Say)	1	1	1	CT, MA, NB, NH, NS, ON, PE, QC, RI
<i>Acilius semisulcatus</i> Aubé	1	1	1	CT, LB, MA, ME, MI, NB, NE, NH, NS, ON, PE, QC, RI, SM
<i>Acilius sylvanus</i> Hilsenhoff	1			MA, ME, NB, NH, NY, ON, PE, QC
<i>Graphoderus liberus</i> (Say)	1	1		CT, MA, ME, NB, NE, NH, NS, ON, PE, QC, RI, SM
<i>Graphoderus perplexus</i> Sharp*	1		1	LB, MA, ME, NB, NE, NH, NS, ON, PE, QC, SM
Dytiscini				
<i>Dytiscus dauricus</i> Gebler*	1	1	1	LB, ME, MI, NB, NE, NS, NY, ON, PE, QC, SM
<i>Dytiscus fasciventris</i> Say†			1	CT, LB, ME, NB, NH, NS, ON, PE, QC, RI
<i>Dytiscus harrisii</i> Kirby	1	1		CT, LB, ME, NB, NE, NH, NS, NY, ON, PE, QC
<i>Dytiscus verticalis</i> Say	1	1	1	CT, MA, ME, NB, NH, NS, NY, ON, PE, QC, RI
Hydaticini				
<i>Hydaticus aruspex</i> Clark*	1		1	CT, LB, MA, ME, NB, NE, NH, NS, ON, PE, QC
Hydroporinae				
Bidessini				
<i>Liodesus affinis</i> (Say)	1	1	1	CT, ME, NB, NE, NH, NS, ON, PE, QC, RI
Hydroporini				
<i>Boreonectes griseostriatus</i> (DeGeer)*	1		1	LB, ME, MI, NB, NE, NS, ON, PE, QC, RI, SM
<i>Hydrocolus paugus</i> (Fall)	1	1	1	LB, MA, ME, MI, NB, NE, NH, NS, NY, ON, PE, QC, SM
<i>Hydrocolus stagnalis</i> (Gemminger & Harold)	1	1	1	CT, MA, ME, NB, NH, NS, NY, ON, PE, QC

Taxon	Counties			Distribution in northeastern NA
	P	Q	K	
<i>Hydroporus dentellus</i> Fall		1	1	LB, MA, ME, NB, NH, NS, NY, ON, PE, QC
<i>Hydroporus fuscipennis</i> Schaum*	1		1	LB, MA, NB, NF, ON, PE, QC, RI
<i>Hydroporus gossei</i> Larson & Roughley	1		1	ME, NB, NF, NY, ON, PE, QC
<i>Hydroporus niger</i> Say	1	1	1	CT, MA, MI, NB, NF, NH, NS, NY, ON, PE, QC, RI
<i>Hydroporus notabilis</i> LeConte*	1	1		LB, MA, ME, MI, NB, NF, NH, NS, ON, PE, QC, SM
<i>Hydroporus obscurus</i> Sturm*		1	1	LB, NB, NF, NS, ON, PE, QC, SM
<i>Hydroporus signatus</i> Mannerheim	1		1	CT, LB, MA, ME, MI, NB, NF, NH, NS, NY, ON, PE, QC, RI, SM
<i>Hydroporus striola</i> (Gyllenhal)*	1	1	1	LB, ME, MI, NB, NF, NH, NS, ON, PE, QC, RI
<i>Hydroporus tenebrosus</i> LeConte	1	1	1	MA, ME, MI, NB, NH, NS, NF, ON, PE, QC,
<i>Hydroporus tristis</i> (Paykull)*	1		1	LB, ME, MI, NB, NF, NH, NY, ON, PE, QC, RI, SM
<i>Nebrioporus rotundatus</i> (LeConte)	1	1	1	LB, MA, ME, NB, NF, NS, ON, PE, QC, RI
<i>Neoporus carolinus</i> (Fall)	1	1		LB, MA, ME, NB, NF, NH, NS, NY, ON, PE, QC, SM
<i>Neoporus chypealis</i> (Sharp)	1	1	1	ME, NB, NH, NS, NY, ON, PE, QC
<i>Neoporus dimidiatus</i> (Gemminger & Harold)	1	1	1	CT, LB, MA, ME, NB, NF, NH, NS, ON, PE, QC, RI
<i>Neoporus sulcipennis</i> (Fall)	1			NB, NH, NY, ON, PE, QC,
<i>Neoporus undulatus</i> (Say)	1	1	1	CT, LB, MA, ME, MI, NB, NF, NS, NY, ON, PE, QC, RI, SM
<i>Sanfilippodytes pseudovilis</i> (Young)	1			LB, MI, NB, NF, ON, PE, QC
Hygrotini				
<i>Hygrotus compar</i> Fall	1			LB, ME, NB, NF, NH, ON, PE, QC
<i>Hygrotus impressopunctatus</i> (Schaller)*	1	1	1	LB, ME, MI, NB, NF, NH, NS, ON, PE, QC
<i>Hygrotus laccophilinus</i> (LeConte)	1	1	1	ME, NB, NH, NS, ON, PE, QC
<i>Hygrotus picatus</i> (Kirby)	1	1	1	LB, MI, NB, NF, NS, NY, ON, PE, QC, RI
<i>Hygrotus sayi</i> Balfour-Browne	1	1	1	LB, MA, ME, MI, NB, NF, NH, NS, NY, ON, PE, QC, RI, SM, VT
<i>Hygrotus turbidus</i> (LeConte)	1	1	1	MA, MI, NB, NH, NS, NY, ON, PE, QC, RI
Hyphdrini				
<i>Desmopachria convexa</i> (Aubé)	1	1	1	ME, MI, NB, NS, ON, PE, QC, RI
Laccornini				
<i>Laccornis latens</i> (Fall)			1	MA, NB, NH, NS, NY, ON, PE, QC,
Laccophilinae				
Laccophilini				
<i>Laccophilus m. maculosus</i> Say	1	1	1	CT, MA, ME, MI, NB, NH, NS, ON, PE, QC, RI
Totals	67	58	66	

**Notes:** Distributional information is derived from Downie and Arnett (1996), Larson et al. (2000), Majka (2008), Webster (2008), Alarie (2009), Majka et al. (2011), Elder and Abraham (2012), Bousquet et al. (2013), Webster and DeMerchant (2012), and Webster (2016). Regional Distribution: CT, Connecticut; LB, Labrador; MA, Massachusetts; ME, Maine; MI, Magdalen Island Archipelago; NB, New Brunswick; NF, insular Newfoundland; NH, New Hampshire; NS, Nova Scotia; NY, New York; ON, Ontario; PE, Prince Edward Island; QC, Québec; RI, Rhode Island; SM, Saint-Pierre et Miquelon; VT, Vermont.

**Table 4.** Comparison of total number of Hydradephaga species by family in the Maritime Ecozone.

	NB	NS	CBI	PEI	SI	MI	NF
Gyrinidae	19	20	9	12	0	7	10
Haliplidae	14	12	4	7	1	2	4
Dytiscidae	108	89	38	65	9	44	80
Total	141	121	51	84	10	53	94

**Notes:** NB, New Brunswick; NS, Nova Scotia; CBI, Cape Breton Island; PEI, Prince Edward Island; SI, Sable Island; MI, Magdalen Island Archipelago; NF, insular Newfoundland. Information is derived from Larson et al. (2000), Alarie (2009), Majka (2008), Webster (2016), this paper. Information from Newfoundland is provided as a basis of comparison.

*Liodessus affinis* (Say), *Nebrioporus rotundatus* (LeConte), *Neoporus carolinus*, *N. clypealis*, and *N. sulcipennis*, which are generally recognized as species with eastern affinities (Larson et al. 2000; Bousquet et al. 2013).

The composition of the Prince Edward Island fauna reflects that of the Maritime Provinces as a whole. All the species found on the island have also been recorded in New Brunswick (Webster 2016) and all but 13 [*Dineutus assimilis* Kirby, *Haliplus canadensis* Wallis, *Peltodytes tortulosus* Roberts, *Acilius sylvanus*, *Copelatus glyphicus*, *Hydroporus fuscipennis*, *H. gossei*, *H. tristis*, *Hygrotus compar*, *H. turbidus* (LeConte), *Neoporus sulcipennis*, *Rhantus consimilis*, and *Sanfilippodytes pseudovilis*] have also been recorded in Nova Scotia. The absence of these 13 species in the latter province is possibly attributable to a lack of collection effort.

In an examination of the Hydradephaga of insular portions of Atlantic Canada (Table 4), we found that despite significantly different land areas, and different distances to the neighbouring continental mainland, the island faunas of Prince Edward Island (with a land area of 5,660 km<sup>2</sup> and 13 km from the mainland) and insular Newfoundland (with a land area of 111,390 km<sup>2</sup>, 18 km distant from Labrador and 110 km from Cape Breton Island) are very similar in the number of species (84 and 94 species respectively) despite differences in composition. The fauna of Cape Breton Island, however, (with a land area of 10,311 km<sup>2</sup> and 1.5 km from the mainland) was 39% less diverse than that of Prince Edward Island consisting of 53 species. In view of the results obtained in this survey, this difference likely could be due to the comparative lack of collecting efforts on Cape Breton Island. In that regard, results from a similar field oriented research on the biodiversity of Hydradephaga of Cape Breton Island (Alarie, in prep.), should add to our knowledge on the faunistic composition of the group in this region.

**Conclusions**

At the light of the many additions made to the faunal list of Prince Edward Island, the preceding account clearly represents a thorough treatment of the aquatic Adephaga of the province. The extensive field oriented research conducted on the island helps at

providing detailed distribution of the Hydradeephaga species in this province as well as detailed habitat information. Whereas additional species could potentially be found, this paper is deemed to represent an accurate account of the faunistic diversity of Hydradeephaga on Prince Edward Island.

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# Species delimitation in the Grayling genus *Pseudochazara* (Lepidoptera, Nymphalidae, Satyrinae) supported by DNA barcodes

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## Abstract

The Palearctic Grayling genus *Pseudochazara* encompasses a number of petrophilous butterfly species, most of which are local endemics especially in their centre of radiation in SW Asia and the Balkans. Due to a lack of consistent morphological characters, coupled with habitat induced variability, their taxonomy is poorly understood and species delimitation is hampered. We employed a DNA barcoding approach to address the question of separate species status for several European taxa and provide first insight into the phylogeny of the genus. Unexpectedly we found conflicting patterns with deep divergences between presumably conspecific taxa and lack of divergence among well-defined species. We propose separate species status for *P. tisiphone*, *P. amalthea*, *P. anymone*, and *P. kermana* all of which have separate well supported clades, with the majority of them becoming local endemics. Lack of resolution in the ‘Mamurra’ species group with well-defined species (in terms of wing pattern and coloration) such as *P. geyeri*, *P. daghestana* and *P. alpina* should be further explored using nuclear molecular markers with higher genetic resolution.

## Keywords

Papilionoidea, Satyrinae, butterflies, phylogeny, barcoding, taxonomy

## Introduction

Depending on which systematic order of classification is adhered to, the genus *Pseudochazara* comprises 27–32 species of Graylings (Gross 1978, Lukhtanov 2007, Savela 2015). It has a wide distribution in the Palearctic region from North Africa to the Himalayas and Mongolia (Tennent 1996, Tshikolovets 2005, Yakovlev 2012). In addition to vague species delimitation, large intraspecific variation has resulted in the description of over 100 subspecific taxa (Lukhtanov 2007) in this intensively studied taxon.

The main reason for the extensive variation in phenotype can be linked with the specific ecological requirements of these butterflies. They are mostly petrophilous and limited to specific rock substrate to which they are perfectly adapted with their camouflaged underside wing pattern and cryptic coloration. Local adaptation to mimic the coloration of the rock substrate is, therefore, one of the main drivers for such large scale diversification (Lorković 1974, Weiss 1980, Hesselbarth et al. 1995, Tennent 1996, but see Anastassiou et al. 2009).

Trying to resolve the systematics of this genus and its species delimitation has been thwarted by the fact that the genitalia of many *Pseudochazara* species are virtually identical and their wing shape and coloration, both being partially dependant on environmental conditions (Gross 1978, Hesselbarth et al. 1995), is inconsistent. The last comprehensive taxonomic review which was published by Gross (1978) is already outdated. He recognised 24 species, among which *P. obscura* (Staudinger, 1878) is now considered a subspecies of *P. lydia* (Staudinger, 1878) (see Eckweiler and Rose 1988), *P. aurantiaca* (Staudinger, 1878) and *P. xerxes* Gross & Ebert, 1975 have been reclassified as subspecies of *P. beroe* (Herrich-Schäffer, 1844) (see Lukhtanov 2007), *P. schahrudensis* (Staudinger, 1881) is now considered conspecific with *P. mamurra* (Herrich-Schäffer, 1844) (see Eckweiler 2004) and *P. pakistana* Gross, 1978 is conspecific with either *P. gilgitica* (Tytler, 1926) (see Lukhtanov 2007) or *P. baldiva* (Moore, 1865) (see Wakeham-Dawson et al. 2007). Several members of the *Pseudochazara* genus from Central Asia that are currently recognised as separate species were considered subspecific taxa in the revision (e.g. *P. droshica* (Tytler, 1926), *P. gilgitica* (Tytler, 1926), *P. lehana* (Moore, 1878)) while *P. euxina* (Kuznetsov, 1909) from Crimea was entirely neglected. Two additional species were described after the revision, *P. kanishka* (Aussem 1980a) and *P. annieae* (Pagès 2007). Following Gross' revision (1978) the shape of the androconial scales of several *Pseudochazara* species has proven to be constant, enabling species delimitation (Weiss 1980, Eckweiler and Rose 1989, Wakeham-Dawson and Kudrna 2000, Wakeham-Dawson et al. 2003, Wakeham-Dawson and Kudrna 2005, Wakeham-Dawson 2006, Wakeham-Dawson and Kudrna 2006, Pages 2007, Wakeham-Dawson et al. 2007).

There has been no attempt to reconstruct the phylogeny of the genus or validate species status using molecular markers. Only the taxonomic position within subtribe Satyrina and a sister relationship to *Chazara* has been established (Peña et al. 2011).

In order to resolve the relationship among *Pseudochazara* species and re-evaluate their species status, in particular of some European taxa, we employed DNA barcoding – using a standardized gene region (5' segment of the mitochondrial gene cytochrome *c* oxidase subunit I = COI) which enabled us to utilize additional *Pseudochazara* sequences available in the Barcode of Life Database (BOLD 2015). DNA barcodes have been widely and successfully used in Lepidoptera taxonomy and species delimitation as an additional set of characters which are independent of habitat conditions (Hebert et al. 2004, Nazari and Sperling 2007, Nazari et al. 2010, Dinca et al. 2011, Yang et al. 2012, Lukhtanov and Novikova 2015, Pazhenkova et al. 2015). However, there are several limitations of this method (see e.g. Wiemers and Fiedler 2004, Brower 2006, Ritter et al. 2013, Song et al. 2008, Toews and Brelsford 2012) which should be taken into account in the interpretation of the gene tree.

## Material and methods

### Sample collection, DNA extraction, amplification, sequencing, and alignment

With the aim of achieving consistency, we adopt the nomenclature of the most recent list of *Pseudochazara* species by Lukhtanov (2007). Following the discovery of *Pseudochazara mamurra amymone* in Albania (Eckweiler 2012), we initially sampled all the *Pseudochazara* taxa from the Balkan Peninsula, a hotspot of *Pseudochazara* diversity in Europe (Verovnik et al. 2014, Gascoigne-Pees et al. 2014). We then broadened the range of our sampling adding additional species from Turkey and the Middle East, the main areas of *Pseudochazara* diversification. Altogether 27 specimens belonging to 10 species of *Pseudochazara*, for which the barcoding gene COI was successfully amplified, were included in the study (see Appendix 1). All specimens were dried prior to DNA extraction. In addition, we included COI sequences from 81 individuals belonging to 14 species from the BOLD database (BOLD 2015). Only specimens that could be unambiguously identified by the voucher photos were selected. Following the nomenclature guidelines proposed by Lukhtanov (2007) a total of 34 taxa belonging to 20 species were included in the analysis. As outgroups, we added several sequences of the closely related Satyrine genus *Chazara* from GenBank, based on the results of the phylogenetic study of Satyrinae by Peña et al. (2011).

Total genomic DNA was extracted from single legs, following the Mammalian tissue preparation protocol (GenElute Mammalian Genomic DNA miniprep kit from Sigma-Aldrich). For each sample a 657 bp fragment of the first subunit of the mitochondrial gene cytochrome *c* oxidase (COI) was amplified using primers LCO1490 and HCO2198 (Folmer et al. 1994). Amplification followed a standard protocol described in Verovnik et al. (2004). PCR products were visualized on an agarose gel to verify amplification success and sequenced by Macrogen in both directions on an Applied Biosystems 3730xl sequencer.



## Phylogenetic analysis

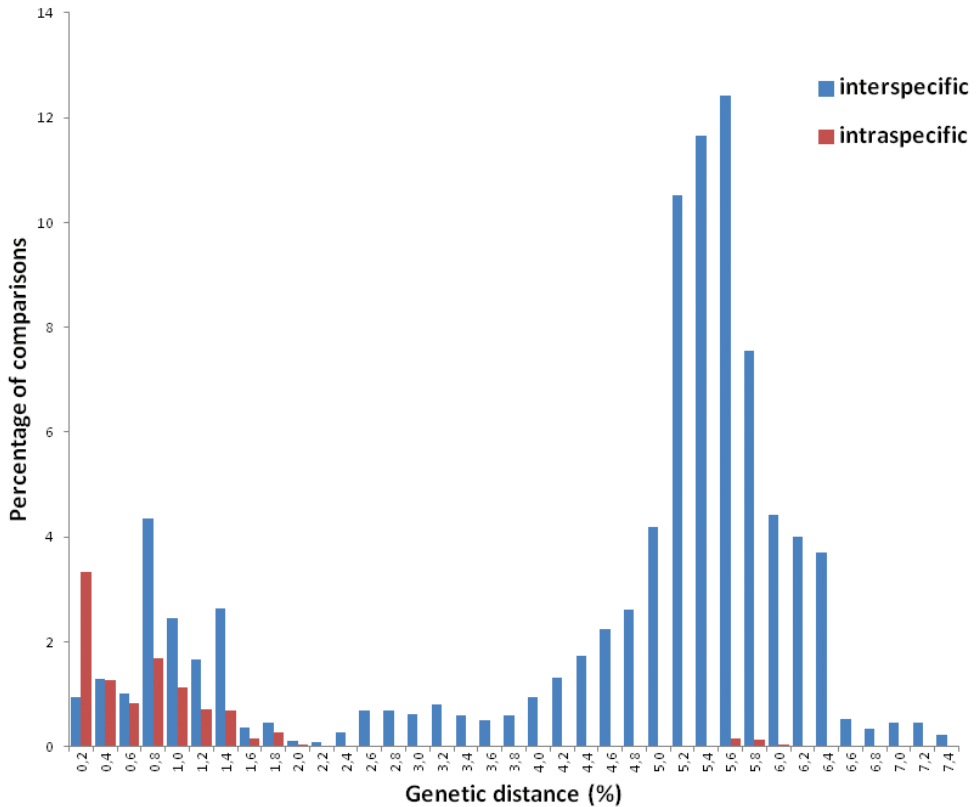
We used Bayesian inference to reconstruct a phylogenetic tree. To achieve more clarity the tree was constructed on a subset of samples including only unique haplotypes belonging to the same taxon. A hierarchical likelihood test was employed in order to test alternative models of evolution, using JModeltest v.0.1.1 (Posada 2008). A GTR (Generalised time reversible) model of nucleotide substitution with gamma distributed rate heterogeneity and a significant proportion of invariable sites was selected in accordance with the Akaike Information Criterion. Bayesian analysis was performed with MrBayes v.3.1.2 implementing the best fit substitution model (Huelsenbeck and Ronquist 2001). Markov chain Monte Carlo search was run with four chains for  $4 \times 10^6$  generations, taking samples every 100 generations. The approximate number of generations needed to obtain stationarity of the likelihood values (“burn-in”) of the sampled trees was estimated graphically to 2000 trees. From the remaining trees posterior probabilities were assessed for individual clades based on their observed frequencies. Trees were visualised using Figtree v.1.4.2 (Rambaut 2014). Genetic distances (p-) were calculated with MEGA 6.0 (Tamura et al. 2013). In addition, a statistical parsimony network analysis was performed with TCS 1.21 (Clement et al. 2000).

## Results

No insertions or deletions were observed in the mitochondrial COI gene and therefore the alignment was unambiguous. For the COI dataset 63 unique haplotypes among 108 *Pseudochazara* sequences were detected. 114 (17.5%) sites were variable and 95 (14.6%) were parsimony informative. The average interspecific genetic distance was 4.9%, but in the case of *P. mnischevii* the intraspecific diversity ranged from 0 to 6.7% with highly distinct divergent sequences of *P. mnischevii tsiaphone*. No evident bar-coding gap was observed separating intraspecific from interspecific pairwise genetic distances (Fig. 1). On the contrary, sharing of identical haplotypes was observed in the following taxa: *P. graeca* / *P. mamurra amymone*, *P. mamurra mamurra* / *P. daghestana*, and *P. beroe aurantiaca* / *P. alpina*. On the other hand, 82% of species comparisons showed high ( $\geq 2\%$ ) interspecific distances.

The calculated maximum connection for parsimony networks at the default 95% limit was 11 steps, and resulted in 9 separate networks within *Pseudochazara*. 6 of them contain only single species (*P. atlantis*, *P. turkestanica*, *P. thelephassa*, *P. lehana*, *P. kanishka*, and *P. anthelea*), whereas the remaining 3 comprise several closely related species (Figs 2–4). Outgroups were contained in 2 distinct networks (*Chazara enervata* and *Chazara briseis*/ *C. heydenreichi*).

The topology of the Bayesian Inference tree of all *Pseudochazara* samples, including the selected outgroup species (Fig. 5), confirms the monophyly of the genus. High posterior probability values support a basal position of *P. atlantis*, the only species of the genus present in (and confined to) North Africa. This is somewhat surprising as

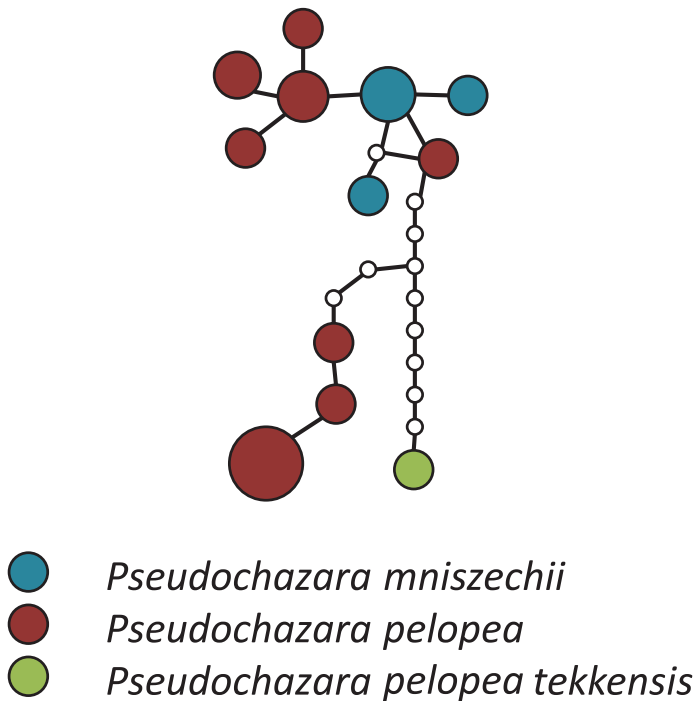


**Figure 1.** Frequency distribution of pairwise intra- and interspecific p-distances of the COI sequences in the genus *Pseudochazara*. No “barcoding gap” exists between these two data series.

*P. anthelea* and *P. thelephassa* are considered to be morphologically the most distinct and separate species within the genus (Gross 1978). *P. atlantis* has tentatively been placed into two groups, the ‘*mamurra*’ species group (Brown 1976), based on androconia shape, and the ‘*pelopea*’ species group (Wakeham-Dawson and Dennis 2001), on account of the shape of male genitalia. *P. atlantis* is also distinctive according to the TCS analysis and forms a separate network. In addition, the second basal split within *Pseudochazara* is well supported, and, apart from some single species clades, three species groups tentatively named as the ‘*pelopea*’, ‘*hippolyte*’ and ‘*mamurra*’ clades received high support. We present the results for these clades separately:

### ‘*Pelopea*’ group

This group, which forms a distinct network in the TCS analysis (Fig. 2), includes two species, *P. pelopea* and *P. mniszechii*. However, there is no genetic differentiation between them, with *P. pelopea persica* and *P. pelopea caucasica* intermixed with *P. mnisze-*

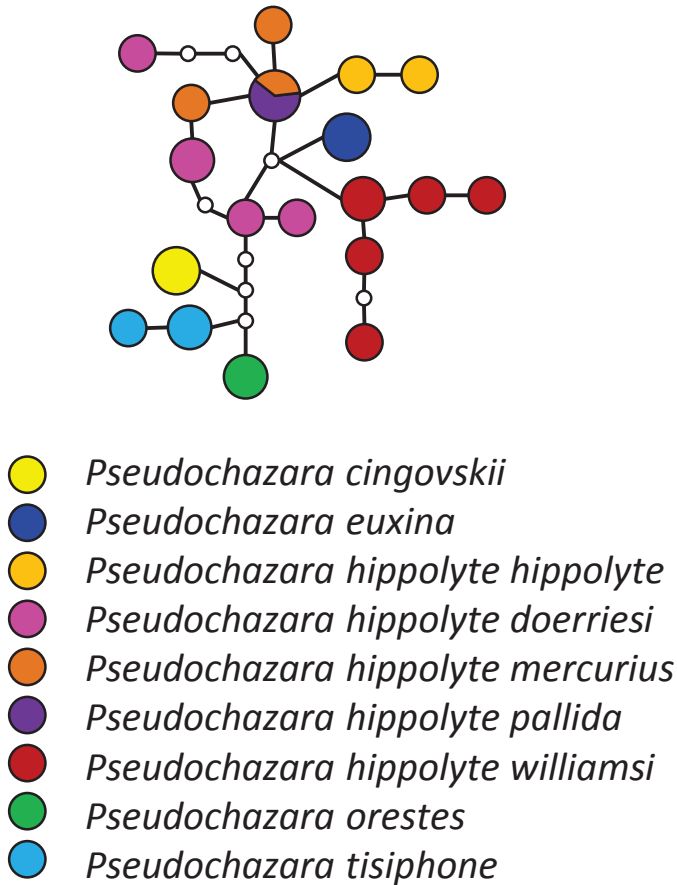


**Figure 2.** Statistical Parsimony network of the ‘pelopea’ species group. Coloured circles represent COI haplotypes and their size corresponds to the number of samples per haplotype. Small white circles represent unsampled haplotypes.

*chii*. Two well supported clades pertain to geographically isolated subspecies of *P. pelopea*, the Levant region (nominotypic *P. pelopea pelopea*) and Kopet Dhag in NE Iran (*P. pelopea tekkensis*). Both subspecies are morphologically distinct from *P. pelopea persica*, in particular the latter, with much wider and more pronounced orange submarginal bands on their forewings. *P. pelopea tekkensis* is considered a separate species by Nazari (2003). *P. mnischevii* is also polyphyletic due to the separate position of the subspecies *tisiphone* from the southern Balkans, which is clearly not closely related, and belongs to the ‘*hippolyte*’ group.

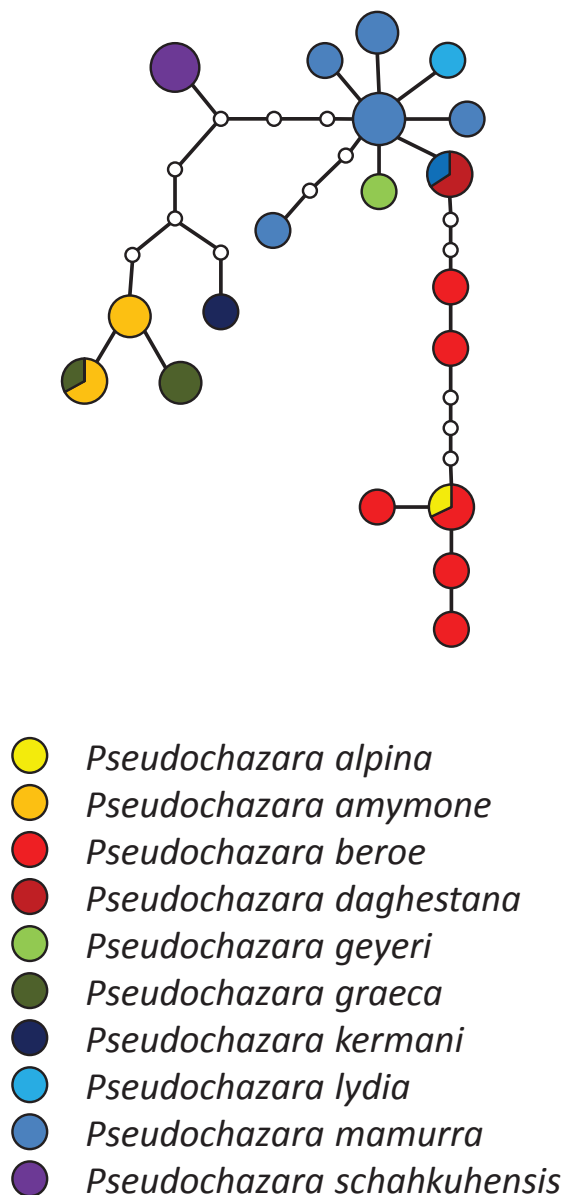
### ‘*Hippolyte*’ group

The ‘*hippolyte*’ clade *sensu stricto* includes the widely distributed *P. hippolyte* complex which has a vast range from southern Spain to central China (Tshikolovets 2011) together with a number of local endemics from the southern Balkan Peninsula: *P. cingovskii* in the Republic of Macedonia, *P. orestes* from north-eastern Greece and the neighbouring part of Bulgaria, *P. mnischevii tisiphone* from north-western Greece and southern Albania and *P. euxina* from the Crimean Peninsula. Both, the haplotype net-



**Figure 3.** Statistical Parsimony network of the 'hippolyte' species group. Coloured circles represent COI haplotypes and their size corresponds to the number of samples per haplotype. Small white circles represent unsampled haplotypes.

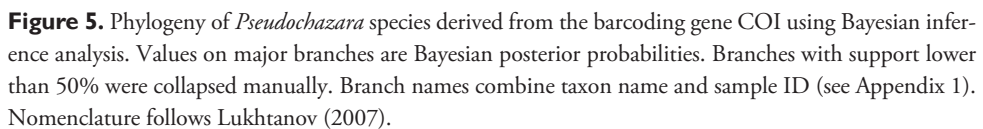
work analysis (Fig. 3) and the phylogeny (Fig. 5) show that *P. mnischechii tisiphone* is not a subspecies of *P. mnischechii* despite superficial resemblance in wing patterns and coloration. In fact, it is closely related to two other local endemics from the Balkan Peninsula, *P. cingovskii* and *P. orestes*. The presence of *P. mnischechii tisiphone* in the western part of Turkey, near Bursa (Hesselbarth et al. 1995) remains to be verified. The single haplotype of *P. euxina* is nested among samples of *P. hippolyte*, so our preliminary results do not support its current status as a separate species. Within this clade *P. hippolyte williamsi* from southern Spain appears basally, however with low posterior probability and it is not monophyletic. All other described subspecies (*P. hippolyte pallida*, *P. hippolyte doerriesi*, *P. hippolyte mercurius*) are less distinct from the nominotypical subspecies, with two Central Asiatic subspecies (*P. hippolyte pallida*, *P. hippolyte mercurius*) sharing haplotypes.



**Figure 4.** Statistical Parsimony network of the ‘mamurra’ species group. Coloured circles represent COI haplotypes and their size corresponds to the number of samples per haplotype. Small white circles represent unsampled haplotypes.

The sister relationship of *P. thelephassa* and *P. anthelea*, which is indicated by genital morphology (the presence of a distinct costal process on the dorsal side of the valve) and wing pattern (the presence of a well-defined black area in the forewing discal cell)





(Aussem 1980b, Hesselbarth et al. 1995, Wakeham-Dawson and Dennis 2001), could not be corroborated as *P. anthelea* appears to be a sister clade to the ‘*hippolyte*’ group *sensu strictu* with high posterior probability. *P. kanishka* from Tajikistan is a sister species of the *anthelea-hippolyte* clade, while *P. thelephassa* is sister taxon to the *anthelea-hippolyte-kanishka* clade, however, with low support. These results concur with wing pattern, i.e. a well-defined black area in the forewing discal cell, also present in specimens of *P. kanishka*.

It is important to note that the average genetic distance between two geographically separated subspecies, *P. anthelea anthelea* from Asia Minor and neighbouring islands, and *P. anthelea amalthea* from the Balkan Peninsula was 1.5%. This result is indicative for differentiation into distinct species as predicted by Kudrna et al. (2011).

In the TCS analysis, this group is split into 3 networks: a) the *hippolyte* clade *sensu stricto* (Fig. 3), b) *P. anthelea*, and c) *P. thelephassa*.

### ‘*Mamurra*’ group

The only two entirely Central Asian species available for analysis, *P. turkestanica* and *P. lehana*, form a well-supported clade together with the ‘*mamurra*’ group, indicating their close relationship, but with a separate network for each in the TCS analysis. All other sequences form a single network (Fig. 4). Although the species sampling in Central Asia is incomplete, there is no evidence of a deep split between Asiatic and European/African taxa as predicted by Wakeham-Dawson and Dennis (2001). The ‘*mamurra*’ group is monophyletic, and includes several well-defined species (in terms of wing patterns, androconia and genitalia) with identical or very similar haplotypes. The following taxa could not be distinguished based on COI haplotypes as they do not form separate monophyletic clades: *P. mamurra*, *P. beroe*, *P. geyeri*, *P. daghestana*, *P. alpina*, and *P. lydia*. Only a single sequence was obtained for *P. geyeri* and *P. lydia*, so their position within this group is tentative. However, it is clear that *P. lydia* is closely related to *P. mamurra* with which it shares similarities e.g. the shape of the androconia (Wakeham-Dawson 2005). *P. alpina* shares the haplotype with *P. beroe* and they appear closely related, however, this is again based on the inclusion of a single sequence.

Within the ‘*mamurra*’ group the only well supported clade includes the taxa *P. schabkuhensis*, *P. mamurra kermana*, *P. graeca* and *P. mamurra amymone*. While *P. schabkuhensis* is sympatric in part of its range with *P. mamurra*, all other taxa have geographically isolated ranges. *P. graeca* and *P. mamurra amymone* are present in the southern part of the Balkan Peninsula with partial range overlap (Pamperis 2009). Both species are clearly morphologically distinct, but genetically not identifiable in COI haplotypes. Clearly this relationship puts in question the status of *P. mamurra amymone* as a subspecies of *P. mamurra*. The same conclusion can be drawn for *P. mamurra kermana* from Iran (Kerman province), which is also well placed within this clade as a sister species to both southern Balkan Peninsula taxa.

## Discussion

Our study supports the monophyly of the genus *Pseudochazara* with high posterior probability values of the COI gene tree. Within the genus, however, two conflicting patterns appear with, unexpectedly, deep divergences between presumably conspecific taxa on the one hand and lack of divergence among well-defined species on the other. This is to some extent concordant with similar studies in related genera in the subfamily Satyrinae (Kodandaramaiah and Wahlberg 2009, Nazari et al. 2010, Kreuzinger et al. 2014). The basal position of *P. atlantis* from North-western Africa as sister group to all remaining *Pseudochazara* species falls into the first category. Based on distinct male genitalia morphology and wing shape/patterns *P. anthelea* and *P. thelephassa* were considered to form the basal split within the genus (Gross 1978, Aussem 1980b, Hesselbarth et al. 1995, Wakeham-Dawson and Dennis 2001). The basal position of *P. atlantis* is difficult to explain in terms of biogeography, as it indicates a North African origin of the genus, which has its centre of divergence much further eastwards in the Middle East (Hesselbarth et al. 1995, Tshikolovets 2011). *P. atlantis* is an alpine species distributed only in the Atlas Mountains of Morocco (Tennent 1996), therefore its isolation from the main distribution of the genus could possibly have preceded the last land bridge connections with Europe at the end of the Miocene (Garcia-Castellanos et al. 2009). Hence, its basal position could be an artefact of long-branch attraction (Bergsten 2005) and/or incomplete sampling of the entirely Asiatic species. Therefore, confirmation with additional genetic markers and additional sampling is required.

Another unexpected result is a deep split between *P. mniszechii* and *P. mniszechii tisiphone*, species which are very similar in wing patterns/coloration and considered conspecific in current literature (Hesselbarth et al. 1995, Kudrna et al. 2011, Tshikolovets 2011, Eckweiler 2012) and databases (Lukhtanov 2007, Savela 2015, Fauna Europaea 2016). Based on the COI gene tree *P. tisiphone* Brown, 1980 (stat. n.) is a separate species closely related to two local endemics from the southern part of the Balkan Peninsula, *P. orestes* and *P. cingovskii*. Actually *P. tisiphone* was originally described as a subspecies of *P. cingovskii* (Brown 1980) and its close relationship was hypothesised also by Wakeham-Dawson and Dennis (2001) based on the similarity of the male genitalia. The low level of genetic differentiation between *P. tisiphone*, *P. orestes*, and *P. cingovskii* indicates a relatively recent speciation, however, we are inclined towards supporting their separate species status based on constant differences in wing patterns/coloration and also their ecological specialization (Pamperis 2009, Verovnik et al. 2013).

A split between *P. anthelea anthelea* from Asia Minor and *P. anthelea amalthaea* from the Balkan Peninsula has been suggested based on minor differences in male genitalia and consistent differences in female wing coloration between both taxa (Olivier 1996, Wakeham-Dawson and Dennis 2001). They are considered separate morphospecies by Kudrna et al. (2011). We can agree with separate species status as the split between the two taxa is much older compared to almost no differentiation in three morphologically and ecologically well defined species: *P. tisiphone*, *P. orestes*, and *P. cingovskii*. Following

this reasoning, *P. pelopea tekkensis* from NE Iran could also be considered a distinct species, however, inclusion of more samples is needed to confirm this status.

Given the high resolution of the basal clades within the COI gene tree, the lack of differentiation between taxa within the ‘*mamurra*’ and ‘*pelopea*’ group was unexpected. In particular, species like *P. geyeri* and *P. daghestana* are among the most easily recognisable species in the genus with uniform and very distinct wing patterns/coloration. There are several possible hypotheses to explain this lack of differentiation:

- Incomplete lineage sorting: recent speciation could result in unresolved relationships among these closely related species; however, well-defined species borders in terms of constant wing pattern differentiation coupled with broad overlaps in species ranges challenges this hypothesis.
- Recent gene flow: gene flow between closely related taxa is a known phenomenon (Descimon and Mallet 2009) and masks relationships among species especially with mitochondrial DNA (Gompert et al. 2008). The species involved have broadly overlapping ranges and could sometimes be found syntopic (Aussem 1980c, Hesselbarth et al. 1995), so hybridization is possible. Actually hybridization is documented even among the most distantly related species such as *P. anthelea* and *P. geyeri* (Aussem, 1980c). Nuclear markers with higher genetic resolution (e.g. microsatellites, SNPs) would be required to study the contact zones between these taxa to confirm ongoing gene flow. It must be noted that partial exclusion is evident when two or more *Pseudochazara* species are syntopic, as one is always dominant, while the others appear in very low frequencies (Hesselbarth et al. 1995, Verovnik et al. 2014).
- Pseudogenes or *Wolbachia* infections: both are common in invertebrates, particularly in arthropods (Bensasson et al. 2011, Gerth et al. 2014, Leite 2012, Ritter et al. 2013). As the vast majority of the haplotypes in the ‘*mamurra*’ and ‘*pelopea*’ clades originate from the BOLD database it is impossible to check or correct for this potential error.

The most enigmatic taxon among the ‘*mamurra*’ group is *P. mamurra amymone* from northern Greece and Albania (Eckweiler 2012, Verovnik et al. 2014). Apart from the author’s original description (Brown 1976) little has been published regarding this elusive taxon for a long time. Failed attempts to locate the vaguely described type locality (Cuvelier 2010) have led to several misleading hypotheses, resulting in speculation that it may even be a rare hybrid between *P. tisiphone* and *P. anthelea* (Wakeham-Dawson and Dennis 2001, Kudrna et al. 2011). Somewhat surprisingly, the COI gene tree suggests it has a close relationship with *P. graeca*, another species from the southern Balkan Peninsula. These two taxa have distinct and constant wing patterns and differ in their habitat requirements, with *P. mamurra amymone* inhabiting steep and hot rocky gorges at lower elevations (Gascoigne-Pees et al. 2014) while *P. graeca* is predominantly a montane (high elevation) species endemic to Greece (Anastassiou et al. 2009). Thus, despite paraphyly of *P. amymone* Brown, 1976 (stat. n.) in relation to *P. graeca*, we believe they both represent valid species within the ‘*mamurra*’ group. Consequently *P. kermana* Eckweiler, 2004

(stat. n.), sister species to *P. amymone* and *P. graeca* combined, should also be elevated to species rank, although additional populations of *P. mamurra* in Iran should be examined to confirm this status. Alternatively, all the taxa within the ‘*mamurra*’ group, including the monophyletic *P. schakuhensis*, a sister species to the *amymone-graeca-kermana* clade, should be treated as a single very polymorphic species, a rather more destructive approach given the current taxonomy.

Although we are aware of the pitfalls of using single gene trees in the interpretation of phylogenetic patterns (Nichols 2001), we believe that strongly supported basal branching and splits between taxa, considered conspecific, represent valid insights into speciation in the *Pseudochazara* genus and together with distinct morphology and ecology allows species delimitation. Hence, we propose separate species status for the following taxa: *P. tisiphone*, *P. amalthea*, *P. amymone*, and *P. kermana*. This has important conservation implications, as most of these species are local endemics and therefore potentially threatened (Verovnik et al. 2014). Wider taxon sampling and inclusion of nuclear markers would undoubtedly help to a better understanding of the taxonomy of this fascinating butterfly genus.

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## Appendix I

Table I. List of samples of the genus *Pseudochazara* included in the barcoding analysis (either own samples with "LA" ID or from BOLD).

ID	GenBank	Species	Location	Lat	Long	Date	Legit
LA16	KU499958	<i>Pseudochazara mamura anymone</i>	Baboshice, Korce, Albania	40°31.038'N	20°47.647'E	11.vii.2012	Rudi Verovnik
LA17	KU499959	<i>Pseudochazara miniszechii tisiphone</i>	Baboshice, Korce, Albania	40°31.038'N	20°47.647'E	11.vii.2012	Rudi Verovnik
LA19	KU499960	<i>Pseudochazara mamura anymone</i>	Devoll Gorge, Korce, Albania	40°42.576'N	20°31.446'E	10.vii.2012	Rudi Verovnik
LA24	KU499961	<i>Pseudochazara cingoskii</i>	Plevar Pass, Prilep, Macedonia	41°22.456'N	21°38.805'E	14.vii.2010	Rudi Verovnik
LA28	KU499962	<i>Pseudochazara miniszechii</i>	Sivas, Turkey	39°41.519'N	36°59.877'E	22.vii.2009	Tarkan Soyhan
LA29	KU499963	<i>Pseudochazara miniszechii</i>	Eskişehir, Turkey	39°43.801'N	30°31.428'E	16.vi.2007	Tarkan Soyhan
LA75	KU499964	<i>Pseudochazara geyeri occidentalis</i>	Galitsia Pass, Macedonia	40°57.379'N	20°48.961'E	30.vii.2013	Filip Franeta
LA76	KU499965	<i>Pseudochazara orestes</i>	Falakro Mt., Greece	41°16.138'N	24°3.947'E	7.vii.2013	Filip Franeta
LA77	KU499966	<i>Pseudochazara graeca</i>	Katara Pass, Metsova, Greece	39°47.580'N	21°12.272'E	22.vii.2012	Filip Franeta
LA78	KU499967	<i>Pseudochazara orestes</i>	Granitits, Drama, Greece	41°18.533'N	23°54.862'E	27.vii.2013	Rudi Verovnik
LA79	KU499968	<i>Pseudochazara graeca</i>	Katara Pass, Metsova, Greece	39°47.580'N	21°12.272'E	26.vii.2013	Rudi Verovnik
LA80	KU499969	<i>Pseudochazara miniszechii tisiphone</i>	Drenovë, Korce, Albania	40°35.352'N	20°48.508'E	21.vii.2013	Rudi Verovnik
LA81	KU499970	<i>Pseudochazara miniszechii tisiphone</i>	Drenovë, Korce, Albania	40°35.352'N	20°48.508'E	21.vii.2013	Rudi Verovnik
LA82	KU499971	<i>Pseudochazara pelopea</i>	Mt. Hermon, Israel	33°19.766'N	35°47.243'E	2013	Dubi Benyamini
LA83	KU499972	<i>Pseudochazara pelopea</i>	Mt. Hermon, Israel	33°19.766'N	35°47.243'E	2013	Dubi Benyamini
LA84	KU499973	<i>Pseudochazara cingoskii</i>	Plevar Pass, Prilep, Macedonia	41°22.456'N	21°38.805'E	2013	Filip Franeta
LA85	KU499974	<i>Pseudochazara cingoskii</i>	Plevar Pass, Prilep, Macedonia	41°22.456'N	21°38.805'E	2013	Filip Franeta
LA86	KU499975	<i>Pseudochazara anthelaea analthea</i>	Veles, Topolka, Macedonia	41°41.915'N	21°46.927'E	2010	Filip Franeta
LA87	KU499976	<i>Pseudochazara anthelaea analthea</i>	Mt. Parnassos, Greece	38°31.233'N	22°36.566'E	2010	Filip Franeta
LA88	KU499977	<i>Pseudochazara anthelaea analthea</i>	Drenovë, Korce, Albania	40°35.352'N	20°48.508'E	2013	Filip Franeta
LA89	KU499978	<i>Pseudochazara mamura birgit</i>	Mt. Aladaglar, Turkey	37°47.568'N	35°9.242'E	2006	Filip Franeta
LA90	KU499979	<i>Pseudochazara miniszechii</i>	Mt. Aladaglar, Turkey	37°47.568'N	35°9.242'E	2006	Filip Franeta
LA92	KU499980	<i>Pseudochazara graeca</i>	Mt. Itri, Greece	38°49.333'N	22°16.635'E	1999	Filip Franeta
LA94	KU499981	<i>Pseudochazara mamura anymone</i>	Drenovë, Korce, Albania	40°35.352'N	20°48.508'E	2013	Filip Franeta
LA95	KU499982	<i>Pseudochazara mamura anymone</i>	Devoll Gorge, Korce, Albania	40°42.576'N	20°31.446'E	2013	Filip Franeta
LA97	KU499983	<i>Pseudochazara lydia obscura</i>	Mersin, Turkey	36°57.017'N	34°23.019'E	12.vii.2010	Tarkan Soyhan
LA124	KU499984	<i>Pseudochazara lehana</i>	Saabo Digur La, Ladakh, India	34°10.554'N	77°39.529'E	15.vii.2013	Joseph Verhulst

ID	GenBank	Species	Location	Lat	Long	Date	Legit
BPAL1699–12		<i>Pseudochazara mamura</i>	Azerbaijan: near Shamkir, 1300 m	40.6989	45.8697	31.vii.2011	Tikhonov V.
BPAL1700–12		<i>Pseudochazara mamura</i>	Azerbaijan: near Shamkir, 1300 m	40.6989	45.8697	31.vii.2011	Tikhonov V.
BPAL1703–12		<i>Pseudochazara alpina</i>	Russia: North Ossetia-Alania, rv. Ardon, Skasan, 1850 m	42.6956	43.9989	12.viii.2011	Tikhonov V.
BPAL2136–13		<i>Pseudochazara kanisbka</i>	Tajikistan: Khodra-Mumin Mnt.			26.v.2001	A. Petrov
BPAL2137–13		<i>Pseudochazara kanisbka</i>	Tajikistan: Khodra-Mumin Mnt.			26.v.2001	A. Petrov
BPAL2138–13		<i>Pseudochazara thelephassa</i>	Iran: Char Mahall-o-Bahtiyari, Sahr-e-Kord, 2000 m			28.v.2002	P. Hofmann
BPAL2139–13		<i>Pseudochazara thelephassa</i>	Iran: Kerman, Kuh-e-Madvar, 5 km S Jowzan, 2400–2600 m			24.v.2002	P. Hofmann
BPAL2140–13		<i>Pseudochazara thelephassa</i>	Iran: Kerman, Kuh-e-Segoch, Mahan Pass, 2400–2600 m			21.v.2002	P. Hofmann
BPAL2141–13		<i>Pseudochazara dagestana savalanica</i>	Iran: Azarbaijan-e-Sharqi, N Taran, Kuh-e-Sabalan, 2900–3000 m			10.vii.2001	Westphal
BPAL2142–13		<i>Pseudochazara dagestana savalanica</i>	Iran: Azarbaijan-e-Sharqi, N Taran, Kuh-e-Sabalan, 2900–3000 m			10.vii.2001	Westphal
BPAL2145–13		<i>Pseudochazara hippolyte mercurius</i>	China: Xinjiang, Tian Shan, Borohoro Shan, 40 km SSW Kyrun, 1850–2050 m	44.0939	84.7942	08.vii.2006	Grieshuber
BPAL2147–13		<i>Pseudochazara mamura kermana</i>	Iran: Kerman, Kuh-e-Madvar, 5 km S Jowzan, 2200–2400 m			28.v.1999	P. Hofmann
BPAL2152–13		<i>Pseudochazara shablkubensis</i>	Iran: Khorasan, Kopet Dag, 15 km E Emam Qoli, N Quchan, 2100–2200 m			19.vi.2001	P. Hofmann
BPAL2153–13		<i>Pseudochazara shablkubensis</i>	Iran: Khorasan, Kopet Dag, Qoucan, 1800 m			13.vii.2000	Hacz-Köszegi
BPAL2154–13		<i>Pseudochazara shablkubensis</i>	Iran: Khorasan, Kopet Dag, Qoucan, 1800 m			14.vii.2000	Hacz-Köszegi
BPAL2155–13		<i>Pseudochazara shablkubensis</i>	Iran: Khorasan, Kopet Dag, Qoucan, 1800 m			15.vii.2000	Hacz-Köszegi
BPAL2156–13		<i>Pseudochazara mamura schabrudensis</i>	Iran: Tehran, Elburs, Tuchal, 2400–2600 m			16.vi.2001	P. Hofmann
BPAL2158–13		<i>Pseudochazara mamura schabrudensis</i>	Iran: Tehran, Elburs, Tuchal, 2400–2600 m			16.vi.2001	P. Hofmann
BPAL2159–13		<i>Pseudochazara mamura schabrudensis</i>	Iran: Tehran, Elburs, Tuchal, 2400–2600 m			16.vi.2001	P. Hofmann

ID	GenBank	Species	Location	Lat	Long	Date	Legit
BPAL2160-13		<i>Pseudochazara mamurra mamurra</i>	Turkey: Artvin, Kilickaya, 1100–1200 m			01.vi.1998	P. Hofmann
BPAL2162-13		<i>Pseudochazara mamurra mamurra</i>	Turkey: Erzurum, Dikmen, SW Üzümdere, 1300 m			16.vii.1998	P. Hofmann
BPAL2172-13		<i>Pseudochazara mamurra sintenisi</i>	Turkey: Bayburt, 5 km N Bayburt, 1500 m			10.vii.1998	P. Hofmann
BPAL2173-13		<i>Pseudochazara mamurra sintenisi</i>	Turkey: Erzincan, 5 km SE Caglayan, 1400 m			08.vii.1998	P. Hofmann
BPAL2174-13		<i>Pseudochazara mamurra sintenisi</i>	Turkey: Gümüşhane, Demirkaynak, 13 km SW Torul, 1100 m			06.vii.1998	P. Hofmann
BPAL2175-13		<i>Pseudochazara mnisechii caucasica</i>	Turkey: Bayburt, 5 km N Bayburt, 1500 m			10.vii.1998	P. Hofmann
BPAL2176-13		<i>Pseudochazara mnisechii caucasica</i>	Turkey: Erzincan, 5 km SE Caglayan, 1400 m			08.vii.1998	P. Hofmann
BPAL2177-13		<i>Pseudochazara mnisechii caucasica</i>	Turkey: Erzurum, road Bayburt-Ispir, Laleli, 1300–1400 m			11.vii.1998	P. Hofmann
BPAL2178-13		<i>Pseudochazara pelopea persica</i>	Iran: Char Mahall-o-Bahitvari, Sahr-e-Kord, 2000 m			28.v.2002	P. Hofmann
BPAL2179-13		<i>Pseudochazara pelopea persica</i>	Iran: Kerman, Kuh-e-Madvar, 5 km S Jowzan, 2400–2600 m			24.v.2002	P. Hofmann
BPAL2180-13		<i>Pseudochazara pelopea persica</i>	Iran: Kerman, Kuh-e-Madvar, 5 km S Jowzan, 2400–2600 m			24.v.2002	P. Hofmann
BPAL2181-13		<i>Pseudochazara pelopea tekkensis</i>	Iran: Khorasan, Kopet Dagħ, 15 km E Eman Qoli, N Quchan, 2100–2200 m			19.vi.2001	P. Hofmann
BPAL2182-13		<i>Pseudochazara berne auranitiaca</i>	Iran: Tehran, Elburs, 15 km NE Firuzkuh pass, 1300–2400 m			24.vii.2000	P. Hofmann
BPAL2183-13		<i>Pseudochazara berne auranitiaca</i>	Iran: Mazandaran, Khosh-Yeylaq, 65 km NE Shahrud, 2000–2100 m			23.vi.2001	P. Hofmann
BPAL2185-13		<i>Pseudochazara berne auranitiaca</i>	Iran: Khorasan, Kopet Dagħ, 15 km E Eman Qoli, N Quchan, 2100–2200 m			19.vi.2001	P. Hofmann
BPAL2245-13		<i>Pseudochazara pelopea pelopea</i>	Israel			22.vi.2013	V.A.Lukhranov & A.V.Novikova
BPAL2246-13		<i>Pseudochazara pelopea pelopea</i>	Israel			22.vi.2013	V.A.Lukhranov & A.V.Novikova
BPAL2247-13		<i>Pseudochazara pelopea pelopea</i>	Israel			22.vi.2013	V.A.Lukhranov & A.V.Novikova
BPAL2281-14		<i>Pseudochazara pelopea pelopea</i>	Syria: Bloudan, 1500 m			16.vii.1999	A. Salk

ID	GenBank	Species	Location	Lat	Long	Date	Legit
BPAL2282–14		<i>Pseudochazana pelopea pelopea</i>	Syria: Bloudan, 1500 m			16.vii.1999	A. Salk
BPAL2692–14		<i>Pseudochazana pelopea pelopea</i>	Israel			03.vii.2014	VLukhanov & A. Novikova
BPAL2701–14		<i>Pseudochazana pelopea pelopea</i>	Israel			03.vii.2014	VLukhanov & A. Novikova
BPAL2702–14		<i>Pseudochazana pelopea pelopea</i>	Israel			03.vii.2014	VLukhanov & A. Novikova
BPAL2728–14		<i>Pseudochazana pelopea pelopea</i>	Israel			04.vii.2014	VLukhanov
BPAL2731–14		<i>Pseudochazana pelopea pelopea</i>	Israel			04.vii.2014	VLukhanov
EULEP451–14		<i>Pseudochazana euxina</i>	Ukraine			11.vii.2007	local collector
EULEP452–14		<i>Pseudochazana euxina</i>	Ukraine			11.vii.2007	local collector
EULEP453–14		<i>Pseudochazana euxina</i>	Ukraine			11.vii.2007	local collector
EULEP487–14		<i>Pseudochazana hippolyte hippolyte</i>	Russia	52.65	59.5667	23.vii.1998	K. Nupponen
EULEP488–14		<i>Pseudochazana hippolyte hippolyte</i>	Russia	51.8	57.0833	14.vii.1998	K. Nupponen
EZHBA660–07		<i>Pseudochazana doerriesi</i>	Russia	51.717	94.4	17.vii.2000	Oleg Kosterin
EZHBA661–07		<i>Pseudochazana doerriesi</i>	Russia	51.717	94.4	17.vii.2000	Oleg Kosterin
EZHBA662–07		<i>Pseudochazana doerriesi</i>	Russia	51.717	94.4	17.vii.2000	Oleg Kosterin
EZHBA899–07		<i>Pseudochazana doerriesi</i>	Russia	51.7667	91.9333	30.vi.2004	Oleg Kosterin
EZHBA900–07		<i>Pseudochazana doerriesi</i>	Russia	51.7667	91.9333	30.vi.2004	Oleg Kosterin
EZROM089–08	HQ004207	<i>Chazara briseis</i>	Romania: Transylvania: Suatu	46.783	23.95	16.viii.2006	Dinca Vlad
EZROM4848–08	HQ004205	<i>Chazara briseis</i>	Romania: Transylvania: Suatu	46.799	23.959	16.viii.2006	Dinca Vlad
EZSPM470–09	GU676107	<i>Pseudochazana hippolyte</i>	Spain: Granada: San Juan (Sierra Nevada)	37.094	-3.115	16.vii.2009	Dinca V.
EZSPN732–09	GU676410	<i>Pseudochazana hippolyte</i>	Spain: Granada: Laguna Seca, Hueneja	37.097	-2.97	18.vii.2008	S. Montagud, J. A. Garcia-Alama & J. Garcia
EZSPN733–09	GU676411	<i>Pseudochazana hippolyte</i>	Spain: Granada: Laguna Seca, Hueneja	37.097	-2.97	18.vii.2008	S. Montagud, J. A. Garcia-Alama & J. Garcia
EZSPN735–09	GU676413	<i>Pseudochazana hippolyte</i>	Spain: Granada: Laguna Seca, Hueneja	37.097	-2.97	18.vii.2008	S. Montagud, J. A. Garcia-Alama & J. Garcia

ID	GenBank	Species	Location	Lat	Long	Date	Legit
EZSPN736-09	GU676406	<i>Pseudochazara hippolyte</i>	Spain: Granada: Laguna Seca, Huencia	37.097	-2.97	18.vii.2008	S. Montagud, J. A. Garcia-Alama & J. Garcia
EZSPN791-09	GU676354	<i>Pseudochazara hippolyte</i>	Spain: Granada: North-East Granada province	37.097	-2.97	23.vii.2008	Gil, Felipe
GWOSF831-10	JF850408	<i>Pseudochazara anthela anthela</i>	Cyprus	34.9559	32.9951	05.vi.2010	M. Seizmaier
IRANB276-08		<i>Pseudochazara berce berce</i>	Iran	38.583	44.367	29.vii.2002	Vazrick Nazari
IRANB278-08		<i>Pseudochazara berce berce</i>	Iran	38.583	44.367	29.vii.2002	Vazrick Nazari
IRANB279-08		<i>Pseudochazara berce berce</i>	Iran	37.776	46.445	22.vi.2001	Vazrick Nazari
IRANB285-08		<i>Pseudochazara berce aurantiaca</i>	Iran	36.12	51.2	16.viii.2000	Vazrick Nazari
IRANB292-08		<i>Pseudochazara pelopea persica</i>	Iran	34.603	47.055	01.vii.2001	Vazrick Nazari
LOWA019-06	FJ663351	<i>Chazara enervata</i>	Kazakhstan: Tianshan: Kurdai Pass	43.333	74.95	11.vi.2000	V. Lukhtanov
LOWA021-06	FJ663349	<i>Chazara enervata</i>	Kazakhstan: Tianshan: Kurdai Pass	43.333	74.95	11.vi.2000	V. Lukhtanov
LOWA022-06	FJ663347	<i>Chazara briseis magna</i>	Kazakhstan: Tianshan: Kurdai Pass	43.333	74.95	11.vi.2000	V. Lukhtanov
LOWA024-06	FJ664025	<i>Pseudochazara turkestanica turkestanica</i>	Kazakhstan: Tianshan: Kurdai Pass	43.333	74.95	11.vi.2000	V. Lukhtanov
LOWA150-06	FJ664021	<i>Pseudochazara hippolyte pallida</i>	Russia	50.1	88.417	07.vii.1999	V. Lukhtanov
LOWA315-06	FJ663353	<i>Chazara beydenreichi</i>	Kazakhstan: Ust-Kamenogorsk Region: Kendyrlilik	47.5	85.183	14.vii.1997	V. Lukhtanov
LOWA316-06	FJ663352	<i>Chazara beydenreichi</i>	Kazakhstan: Ust-Kamenogorsk Region: Kendyrlilik	47.5	85.183	14.vii.1997	V. Lukhtanov
LOWA516-06	FJ664024	<i>Pseudochazara turkestanica turkestanica</i>	Kyrgyzstan: Gultcha distr.: Chitkala	39.85	73.333	29.vii.1995	V. Lukhtanov
LOWA517-06	FJ664023	<i>Pseudochazara turkestanica turkestanica</i>	Kyrgyzstan: Gultcha distr.: Chitkala	39.85	73.333	29.vii.1995	V. Lukhtanov
LOWA608-06	FJ663348	<i>Chazara briseis maracandica</i>	Uzbekistan: Kashkardarinskaya obl.: Tamshush	38.967	67.4	20.vi.1994	V. Lukhtanov
LOWA680-06	FJ664020	<i>Pseudochazara hippolyte mercurius</i>	Kazakhstan: Dzhambul'skaya obl.: Kurdai Pass	43.333	74.95	28.vi.1993	V. Lukhtanov
LOWA681-06	FJ664019	<i>Pseudochazara hippolyte mercurius</i>	Kazakhstan: Dzhambul'skaya obl.: Kurdai Pass	43.333	74.95	28.vi.1993	V. Lukhtanov
LOWA787-06	FJ664018	<i>Pseudochazara hippolyte hippolyte</i>	Kazakhstan: Dzhambul'skaya obl.: Kurdai Pass	47.4	83.917	22.vi.1997	V. Lukhtanov
LOWA788-06	FJ664022	<i>Pseudochazara turkestanica turkestanica</i>	Kazakhstan: Dzhambul'skaya obl.: Kurdai Pass	47.4	83.917	22.vi.1997	V. Lukhtanov
LOWA8040-07		<i>Pseudochazara pelopea persica</i>	Armenia	40.083	44.917		Andrei Sourakov
LOWA8041-07		<i>Pseudochazara pelopea persica</i>	Armenia	40.083	44.917		Andrei Sourakov
LOWA8046-07		<i>Pseudochazara pelopea persica</i>	Armenia	40.083	44.917		Andrei Sourakov
LOWA8046-07		<i>Pseudochazara pelopea caucasica</i>	Armenia	40.083	44.917		Andrei Sourakov



ID	GenBank	Species	Location	Lat	Long	Date	Legit
LOWAB047–07		<i>Pseudochazara pelopea persica</i>	Armenia	40.083	44.917		Andrei Sourakov
LOWAB048–07		<i>Pseudochazara pelopea persica</i>	Armenia	40.083	44.917		Andrei Sourakov
WMB1212–13		<i>Pseudochazara atlantis</i>	Morocco	33.025	-5.071	01.vii.2011	Vila, R., Dinca, V. & Voda, R.
WMB1213–13		<i>Pseudochazara atlantis</i>	Morocco	33.025	-5.071	01.vii.2011	Vila, R., Dinca, V. & Voda, R.
WMB2163–13		<i>Pseudochazara atlantis</i>	Morocco	31.09	-7.915	15.vii.2012	Tarrier, Michel

# The collection of birds from São Tomé and Príncipe at the Instituto de Investigação Científica Tropical of the University of Lisbon (Portugal)

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## Abstract

The former Instituto de Investigação Científica Tropical-IICT (Lisbon, Portugal), recently integrated into the University of Lisbon, gathers important natural history collections from Portuguese-speaking African countries. In this study, we describe the bird collection from the Democratic Republic of São Tomé and

Príncipe, which was fully taxonomically checked and georeferenced. The IICT bird collection contains 5598 specimens, of which 559 are from São Tomé and Príncipe, representing 85 taxa, including 19 endemic species and 13 endemic subspecies of birds. The specimens were collected between 1946 and 1973, although 43% of the records are from 1954 and 45% are from 1970. The geographic distribution of samples covers the whole territory, with a higher number of records from São Tomé than from Príncipe. The districts with highest number of records are Pagué (equivalent to Príncipe Island), and Água Grande and Mé-Zochi on São Tomé. Despite the relatively low number of specimens per taxon, the importance of the collection is considerable due to the high number of endemic and threatened species represented. Furthermore, it adds valuable information to the GBIF network, especially for a country whose two islands are each an Endemic Bird Area and for which substantial gaps in ornithological knowledge remain.

### Keywords

Animalia, Aves, Chordata, Gulf of Guinea, Museum, Biodiversity databases, Species Occurrence data, Specimen

## Introduction

The Democratic Republic of São Tomé and Príncipe is a country comprising two oceanic islands (São Tomé and Príncipe) and several islets located *ca.* 200 km from the coast of Gabon in the Gulf of Guinea, West Africa. From a bird dispersal perspective, the islands lie close enough to a biodiversity rich continental coast to make biological colonization likely, but sufficiently distant to allow successful colonizers to evolve in isolation from their mainland counterparts (Melo 2007). As a result, the endemism of bird species supported by each island is remarkable. In relation to their area, the number of endemic bird species is the highest globally and, although this is the second smallest country in Africa (*ca.* 1000 km<sup>2</sup>) it ranks in third place regarding the number of endemic birds (Stattersfield et al. 1998). Its forests have been considered the third most important in the world from a bird conservation perspective (Buchanan et al. 2011).

A total of 88 bird species are recorded for the islands (BirdLife International 2015), although that number increases to about 150 if vagrants are included (e.g., Christy 2001). There are 27 endemic species of which four are classified as Critically Endangered, one as Endangered and seven as Vulnerable (IUCN 2014, BirdLife International 2015; Suppl. material 1: Table S1). Twelve continental African bird species are represented by endemic subspecies (Table 2).

Despite the high numbers of endemic species, studies of the avifauna remained sporadic well into the 20th Century (Jones and Tye 2006). The importance of this unique avifauna was finally brought to the attention of the international conservation community during the 1980s (Collar and Stuart 1985, 1988). Based on the scarce literature available, the forests of São Tomé were ranked as the second most important of Africa and Madagascar from a bird conservation perspective (Collar and Stuart 1985) and seven species were tentatively classified as threatened following the criteria of the International Union for Conservation of Nature (IUCN) (Collar and Stuart 1988). Most importantly, these publications highlighted the worrying lack of up-to-

date knowledge on the avifauna and the urgency in reversing this situation. New expeditions followed this call, rediscovering species not seen for over 60 years, including the São Tomé Grosbeak which was 'lost to science' for 101 years (Jones and Tye 1988, Atkinson et al. 1991, Sergeant et al. 1992). A steady number of research projects have continued since then (e.g., Christy and Clarke 1998, Melo and O'Ryan 2007, Melo and Fuchs 2008, Dallimer et al. 2009, 2010, Melo et al. 2010, 2011, de Lima et al. 2013, 2014).

Historical data from biological collections have played a central role in building-up our knowledge on the country's avifauna (Amadon 1953, Frade 1958, 1959, Frade and Santos 1970, Jones and Tye 2006), especially when collections were made during periods without systematic ornithological surveys (Hromada et al. 2003, 2015, Leventis and Olmos 2009). Additionally, these collections are a source of valuable material for research including: i) taxonomic, phylogenetic and biogeographic studies; ii) diet studies from isotope analysis from feathers or nails; and iii) assessing changes in pollution by measuring heavy metal contents on feather samples collected at different point in the past.

This is the second of a series of data papers dedicated to the bird collection held by the Instituto de Investigação Científica Tropical of the University of Lisbon, following a previous one dedicated to birds from Angola (Monteiro et al. 2014). Here we provide a fully taxonomically revised and georeferenced dataset of the specimens from São Tomé and Príncipe, following the International Ornithological Congress taxonomic nomenclature (IOC World Bird List, v6.1) (Gill and Donsker 2016). The dataset is freely available online on the IICT IPT provider (<http://maerua.iict.pt/ipt>) and on the Global Biodiversity Information Facility (GBIF) data portal (<http://www.gbif.org>). It comprises 559 specimens from 107 different locations on both islands, collected between 1946 and 1973. Most specimens (491) were collected during two scientific expeditions that took place in 1954 and 1970, which were led by the collectors Fernando Frade (IICT) and René de Naurois (French naturalist that collaborated with IICT), respectively.

## **General description**

The bird collection of the Instituto de Investigação Científica Tropical (IICT), of the University of Lisbon holds a total of 5598 specimens, mainly from the Portuguese-speaking African countries: Mozambique, Angola, Guinea-Bissau, São Tomé and Príncipe, and Cape Verde. The dataset described here is the full subset from São Tomé and Príncipe, which contains 559 specimens that were taxonomically revised and georeferenced.

The collection comprises a high number of bird endemisms, including 19 out of 27 known endemic species of the country, together with an additional one shared with Annobón Island (Tables 1, Suppl. material 1: S1), and 13 of the 14 endemic subspecies described for the islands (Tables 2, Suppl. material 1: S2). The IICT collection holds 345 specimens from São Tomé Island, 213 from Príncipe Island and one lacking island information.

**Table 1.** Endemic species of the Republic of São Tomé and Príncipe represented in the IICT collection, including the number of specimens. The taxonomical nomenclature follows the IOC World Bird List v6.1 (Gill and Donsker 2016).

Common Name	Species	São Tomé (N)	Príncipe (N)	IUCN Red List (version 2014)
São Tomé Olive Pigeon	<i>Columba thomensis</i> Barboza du Bocage, 1888	11		Endangered
São Tomé Green Pigeon	<i>Treron sanctithomae</i> (Gmelin, JF, 1789)	7		Vulnerable
São Tomé Spinetail	<i>Zonavena thomensis</i> (Hartert, 1900)	2	2	Least Concern
São Tomé Oriole	<i>Oriolus crassirostris</i> Hartlaub, 1857	6		Vulnerable
São Tomé Paradise Flycatcher	<i>Terpsiphone atrochalybeia</i> (Thomson, 1842)	14		Least Concern
São Tomé Prinia	<i>Prinia mollerii</i> Barboza du Bocage, 1887	9		Least Concern
Dohrn's Thrush-Babbler	<i>Horizorhinus dohrni</i> (Hartlaub, 1866)		8	Least Concern
Príncipe Speirops	<i>Zosterops leucophaeus</i> (Hartlaub, 1857)		3	Near Threatened
Black-capped Speirops	<i>Zosterops lugubris</i> (Hartlaub, 1848)	15		Least Concern
São Tomé White-eye	<i>Zosterops feae</i> Salvadori, 1901	3		NA <sup>1</sup>
Príncipe Starling	<i>Lamprolornis ornatus</i> (Daudin, 1800)		13	Least Concern
São Tomé Thrush	<i>Turdus olivaceofuscus</i> Hartlaub, 1852	10		Near Threatened
Príncipe Sunbird	<i>Anabathmis hartlaubii</i> (Hartlaub, 1857)		11	Least Concern
Newton's Sunbird	<i>Anabathmis newtonii</i> (Barboza du Bocage, 1887)	11		Least Concern
Giant Sunbird	<i>Dreptes thomensis</i> (Barboza du Bocage, 1889)	5		Vulnerable
Príncipe Weaver	<i>Ploceus princeps</i> (Bonaparte, 1850)		14	Least Concern
Giant Weaver	<i>Ploceus grandis</i> (Gray, GR, 1844)	19		Least Concern
São Tomé Weaver	<i>Ploceus sanctithomae</i> (Hartlaub, 1848)	11		Least Concern
Príncipe Seedeater	<i>Crithagra rufobrunnea</i> (Gray, GR, 1862)	13	9	Least Concern

<sup>1</sup>Treated by IUCN as a subspecies of *Zosterops ficedulinus*. Nominate subspecies occurs on Príncipe. Vulnerable status applies to the two populations.

Each island is an independent Endemic Bird Area (Stattersfield et al. 1998), and their forests have been considered the third most important forests in the world for bird conservation, the other two being Hawaii tropical forests and Palau tropical moist forests (Buchanan et al. 2011). Although the original specimen labels lack full collecting information, it is possible to infer that the main collectors were Fernando Frade and René de Naurois. Fernando Frade visited the archipelago in 1954, and René de Naurois in 1970, precisely the years that aggregated 88% of the specimens (43% in 1954 and 45% in 1970).

## Records of special significance

This collection, although relatively small, provides a significant contribution to the ornithology of the islands as it was mainly put together from expeditions that took place



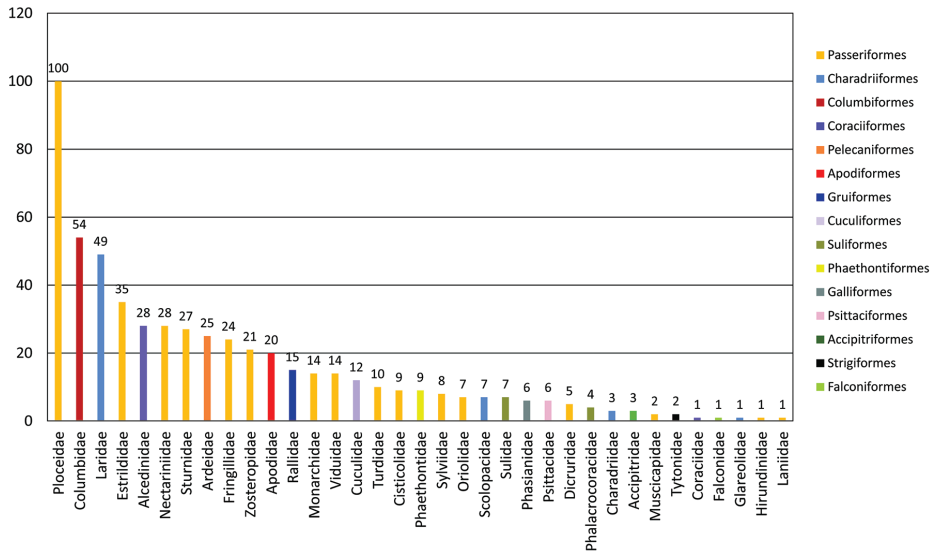
**Table 2.** Endemic subspecies of African continental species present in São Tomé and Príncipe in IICT collection. The taxonomical nomenclature of the species follows the IOC Bird List v6.1.

Common Name	Species	Subspecies			
		São Tomé	N	Príncipe	N
Harlequin Quail	<i>Coturnix delegorguei</i>	<i>histrionica</i> Hartlaub, 1849	6		
Lemon Dove	<i>Columba larvata</i>	<i>simplex</i> (Hartlaub, 1849)	3	<i>principalis</i> (Hartlaub, 1866)	13
African Green Pigeon	<i>Treron calvus</i>			<i>virescens</i> Amadon, 1953	7
African Emerald Cuckoo	<i>Chrysococcyx cupreus</i>			<i>insularum</i> Moreau & Chapin, 1951	11
Western Barn Owl	<i>Tyto alba</i>	<i>thomensis</i> (Hartlaub, 1852)	2		
Little Swift	<i>Apus affinis</i>	<i>bannermani</i> Hartert, 1928	5	<i>bannermani</i> Hartert, 1928	9
Blue-breasted Kingfisher	<i>Halcyon malimbica</i>			<i>dryas</i> Hartlaub, 1854	9
Malachite Kingfisher	<i>Corythornis cristatus</i>	<i>thomensis</i> Salvadori, 1902	14	<i>nais</i> (Kaup, 1848)	5
Velvet-mantled Drongo	<i>Dicrurus modestus</i>			<i>modestus</i> Hartlaub, 1849	5
Chestnut-winged Starling	<i>Onychognathus fulgidus</i>	<i>fulgidus</i> Hartlaub, 1849	7		
Southern Masked Weaver	<i>Ploceus velatus</i>	<i>peixotoi</i> Frade & Naurois, 1964	14		

in periods when otherwise no systematic ornithological surveys took place. In the 20<sup>th</sup> century, the only other main collection efforts took place at the turn of the century and in 1928 (Jones and Tye 2006). The collection comprises specimens from 19 out of the 27 endemic bird species of the oceanic islands of the Gulf of Guinea: 10 single-island endemic species from São Tomé, 5 single-island endemic species from Príncipe, 4 endemic species present on both islands (Table 1) and one species endemic to Príncipe, São Tomé and Annobón islands. The IUCN Red List classifies four of these endemic species as Vulnerable and one as Endangered (Suppl. material 1: Table S1).

The collection also includes specimens from 13 subspecies from African continental species (Table 2). These include five specimens of *Dicrurus modestus* Hartlaub, 1849, which are of particularly interest as they may contribute to settle the long-standing debate on whether the population from Príncipe Island is a separate endemic species (*cf.* Jones and Tye 2006) – both by allowing specimens to be compared with mainland birds and by providing material for genetic analyses.

In addition, the collection is valuable in that it adds information to the existing data available through GBIF in terms of collecting dates and includes specimens of some endemic species for which there are few specimens in the collections worldwide, such as the São Tomé Green Pigeon (*Treron sanctithomae* (Gmelin, JF, 1789)), the São Tomé Olive Pigeon (*Columba thomensis* Barboza du Bocage, 1888) and the São Tomé subspecies of the Southern Masked Weaver (*Ploceus velatus peixotoi* Frade & Naurois, 1964). Finally, considering the size of the collection, it is surprising that it includes several specimens that represent the only known records for the islands: Pectoral Sandpiper (*Calidris melanotos* (Vieillot, 1819)), Great Spotted Cuckoo (*Clamator glandarius* (Linnaeus, 1758)), Red-footed Falcon (*Falco vespertinus* Linnaeus, 1766), Lesser Grey Shrike (*Lanius minor* Gmelin, JF, 1788) and Eurasian Golden Oriole (*Oriolus oriolus* (Linnaeus, 1758)).



**Figure 1.** Total number of specimens per family. The legend lists the corresponding Orders, with assigned colors.

## Taxonomic coverage

The IICT São Tomé and Príncipe bird collection comprises 15 orders and 35 families. The most represented orders are Passeriformes (54.7%), Charadriiformes (10.7%) and Columbiformes (9.7%). The families Ploceidae, Columbidae and Laridae are the ones with the highest number of records (100, 54 and 49, respectively) (Figure 1). The families Hirundinidae, Laniidae, Glareolidae, Falconidae, Coraciidae are each represented by a single record.

## Taxonomic ranks

**Kingdom:** Animalia

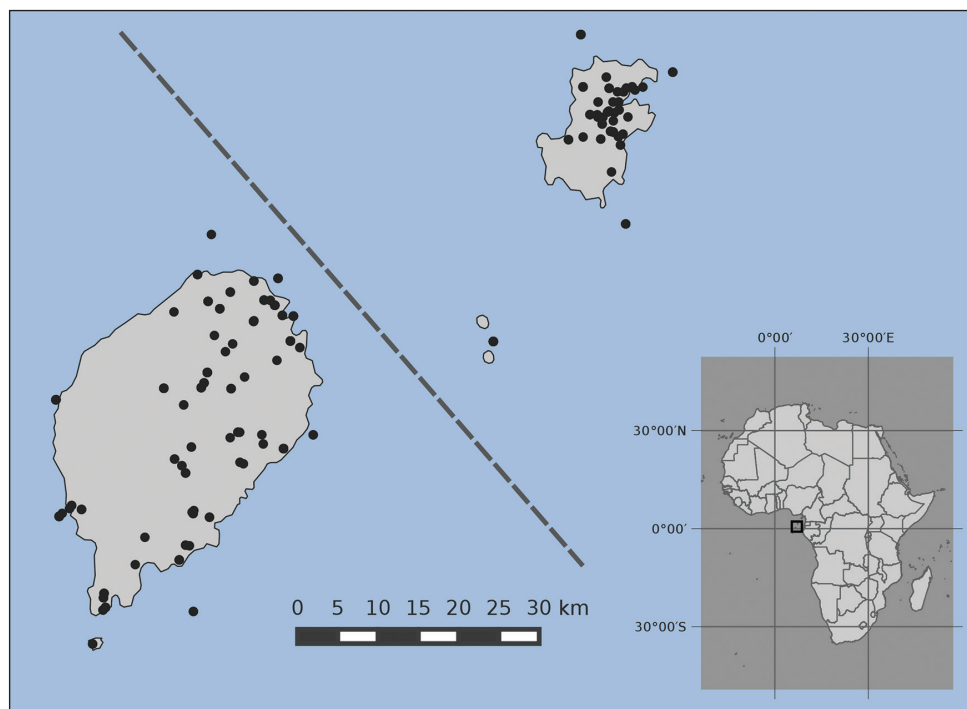
**Phylum:** Chordata

**Class:** Aves

**Order:** Accipitriformes, Apodiformes, Charadriiformes, Columbiformes, Coraciiformes, Cuculiformes, Falconiformes, Galliformes, Gruiformes, Passeriformes, Pelecaniformes, Phaethontiformes, Psittaciformes, Strigiformes, Suliformes

**Family:** Accipitridae, Alcedinidae, Apodidae, Ardeidae, Charadriidae, Cisticolidae, Columbidae, Coraciidae, Cuculidae, Dicruridae, Estrildidae, Falconidae, Fringillidae, Glareolidae, Hirundinidae, Laniidae, Laridae, Monarchidae, Muscicapidae, Nectariniidae, Oriolidae, Phaethontidae, Phalacrocoracidae, Phasianidae, Ploceidae, Psittacidae, Rallidae, Scolopacidae, Sturnidae, Sulidae, Sylviidae, Turdidae, Tytonidae, Viduidae, Zosteropidae

**Common names:** Birds



**Figure 2.** Distribution map of specimens occurrence throughout the territory of São Tomé and Príncipe. To facilitate graphic representation, distances between the two islands are not to scale (indicated by the dashed line).

## Spatial and temporal coverage

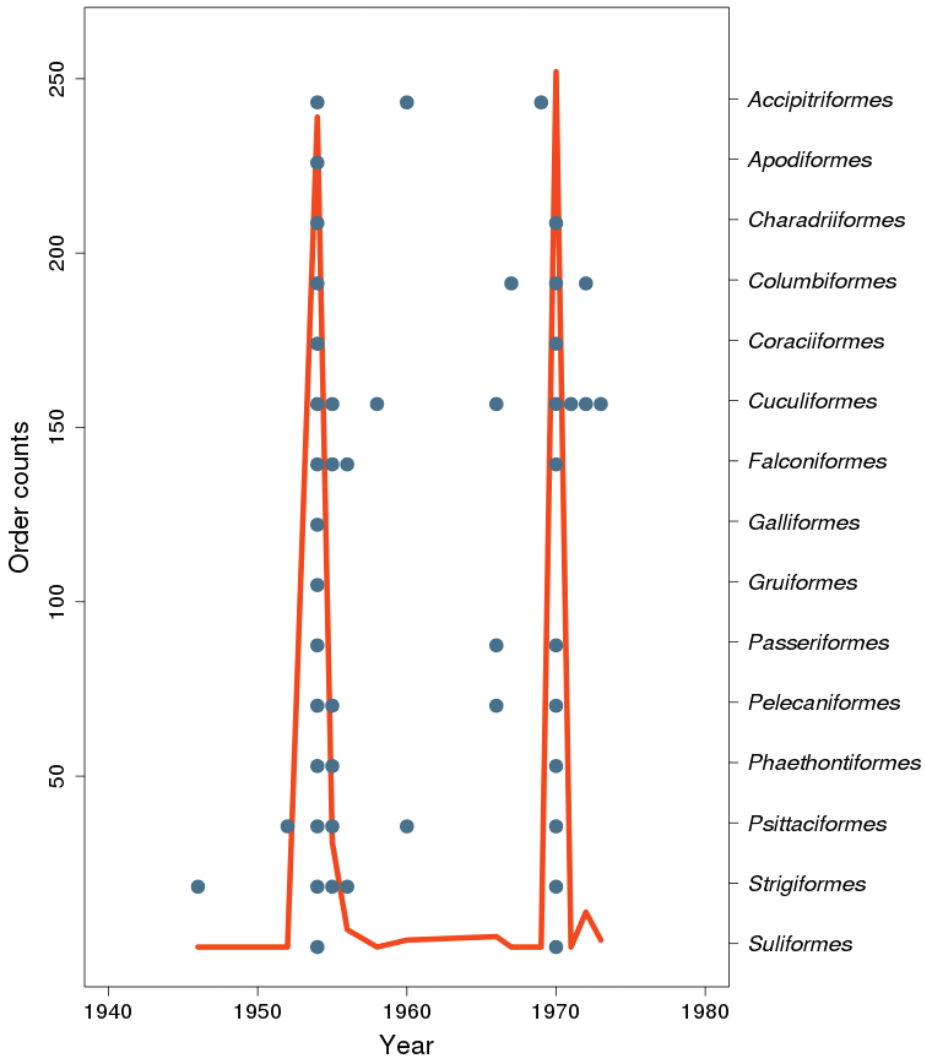
**General spatial coverage:** São Tomé and Príncipe is a island country that consists of two archipelagos around two main islands, São Tomé and Príncipe, located in the equatorial Atlantic, in the Gulf of Guinea, at about 250 km west of the western equatorial coast of Africa. The islands are of volcanic origin, with maximum altitudes of 2024 m and 948 m for São Tomé and Príncipe, respectively.

The geographic range of the collection covers the whole territory of São Tomé and Príncipe (Figure 2). São Tomé Island has 345 records, while Príncipe Island has 213 records. The number of records per each of the seven districts of the country is as follows:

São Tomé Island - Água Grande (87), Mé-Zóchi (75), Caué (54), Lembá (51), Lobata (41) and Cantagalo (34). Príncipe Island - Pagué (213). For three records from São Tomé the district is unknown and for one record both district and island are unknown.

**Coordinates:** São Tomé (0°25'N and 0°01'S Latitude; 6°28'E and 6°45'E Longitude); Príncipe (1°32'N and 1°43'S Latitude; 7°20'E and 7°28'E Longitude).

**Temporal coverage:** The temporal range of the records lies between 1946 and 1973 (Figure 3). Most of the specimens (88%) were collected in two expeditions, which occurred in 1954 and 1970.



**Figure 3.** The sampling temporal profile of the collection's specimens, showing the number of specimens per collection date. Blue dots represent sampling years for each Order.

## Methods

**Method step description:** The collection of birds and mammals of IICT was catalogued for the first time under the project ARCA (2008–2010) using the software Specify Workbench and later imported to the collections' database managed with the software Specify version 6 (Specify Software Project 2013). The catalogued information was transcribed but not revised or updated. Since 2012, the IICT bird collection has been taxonomically revised by the first author, with updates incorporated into the database. The cataloguing and georeferencing procedure followed Monteiro et al. (2014). Taxonomy followed

the IOC Bird List (v6.1) (Gill and Donsker 2016), although the correspondence with taxonomy followed by BirdLife/IUCN is showed in Suppl. material 1: Tables S1 and S2. The information on the labels (collector, date of collecting, locality, descriptions of bill, eye and foot) was re-checked and the database corrected as necessary.

Since there were no geographic coordinates on labels or in associated record books, the georeferencing of specimen localities followed Chapman and Wieczorek (2006). The gazetteer Geolocate (Rios and Bart 2014) and Google Maps, were used to determine the coordinates and their uncertainty. The 1:25000 maps of São Tomé and Príncipe (IICT 1962, 1964) were used to search for coordinates not present on the gazetteers and, when possible, to fine-tune the positions. For four records there was no sufficient information to determine the geographic coordinates. The coordinates are given as decimal degrees using datum WGS 84.

**Study extent description:** The study covers both islands of the Democratic Republic of São Tomé and Príncipe. There are more samples for São Tomé (345) than for the smaller island of Príncipe (213). The best represented districts are Água Grande, Mé-Zochi (São Tomé Island) and Pagué (Príncipe Island).

**Sampling description:** Most of the records of the collection resulted from scientific visits or expeditions carried out between 1946–1973. There were two main collectors, Fernando Frade and René de Naurois, and two main dates 1954 and 1970. In 1954, the director of the Centre of Zoology of the Junta das Missões Geográficas e de Investigações do Ultramar (nowadays IICT-ULisboa), Fernando Frade, coordinated a three months scientific expedition to São Tomé and Príncipe (Missão Científica de São Tomé e Príncipe). In 1970, René de Naurois, visited the islands in one of his scientific trips (1963–1973) to collect and study São Tomé and Príncipe's avifauna. Part of the scientific data gathered from the collected bird specimens were later published in the first book on the birds of the oceanic islands of the Gulf of Guinea (Naurois 1994).

**Quality control description:** For the development of the dataset the data from the labels of each specimen was revised by the first author because, initially, these had been transcribed as verbatim to the Specify 6 database. A taxonomic revision of the scientific names and a data checking were performed using IOC Bird List (version 6.1) followed by georeferencing according to the recommendations of Chapman and Wieczorek (2006), including the determination of uncertainty.

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## **Supplementary material I**

### **Species lists referred in Tables 1 and 2 showing correspondence between scientific and common names between IOC and BirdLife Internaction/IUCN nomenclatures**

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Data type: species lists

Explanation note: Nomenclatural differences between International Ornithological Council and BirdLife International/IUCN lists (2016) for endemic species and endemic subspecies of São Tomé and Príncipe.

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